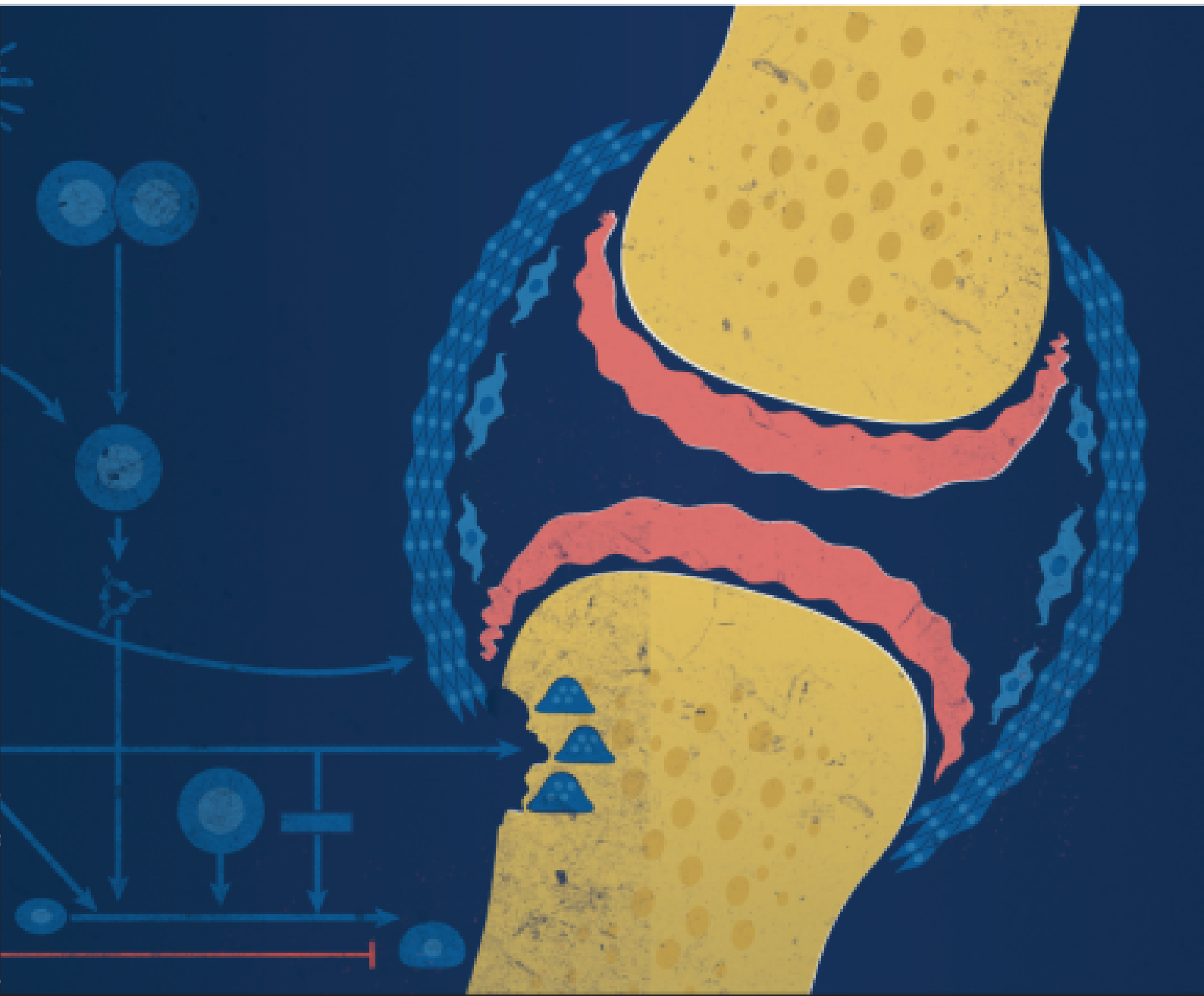
July 2022 volume 18 no. 7
www.nature.com/nrrheum

FOCUS
Immunology of rheumatoid arthritis

nature reviews rheumatology



RHEUMATOID ARTHRITIS
The role of autoreactivity, cellular metabolism, macrophages and more

Mechanisms of joint destruction
Immune cells, fibroblasts and bone

GENETICS

Single-cell RNA sequencing sheds light on cell-type specific gene expression in immune cells

> the studies provide valuable insights into inter-individual differences in immune function at the cellular level

Single-cell RNA sequencing (scRNA-seq) of peripheral blood mononuclear cells (PBMCs) is a promising approach to profile circulating immune cells. The findings of two new studies, each of which involved analysis of more than one million cells from hundreds of individuals, have substantially expanded what we know about how genetic variants influence autoimmune diseases.

In one study, Perez et al. used multiplex scRNA-seq (mux-seq), a method they had previously developed to enable single-cell profiling of large populations, to study more than 1.2 million PBMCs from 162 people diagnosed with systemic lupus erythematosus (SLE) and 99 healthy individuals of either Asian or European descent. "Large sample sizes are particularly important for studying complex autoimmune diseases such as lupus, where patients present with a variety of symptoms and may respond very differently to current treatments," notes corresponding author Chun Jimmie Ye.

In comparison with healthy individuals, patients with SLE had a lower percentage of naive $CD4^+$ T cells and an expansion of cytotoxic $CD8^+$ T cells; these differences were not associated with treatment. Expression of interferon-stimulated genes was increased across 11 cell types in patients with SLE, with monocytes having the most prominent type I interferon signature. The cell type-specific expression profiles obtained with mux-seq could be used to predict case–control status and stratify patients by disease severity.

The researchers then integrated mux-seq data with genotyping data to identify cell type-specific *cis*-expression quantitative trait loci (eQTL) across eight types of immune cells. They found 535 genes with at least one cell type-specific *cis*-eQTL and 1,207 *cis*-eQTLs shared across all cell types.

Integration of genome-wide association study data with *cis*-eQTLs provided insights into which cell types were relevant to disease heritability, and using cell type-specific *cis*-eQTLs in a modified transcriptome-wide association study identified 73 novel genetic associations with SLE. "We are now using mux-seq to profile other autoimmune diseases in order to understand how related diseases share molecular and compositional signatures," says Ye.

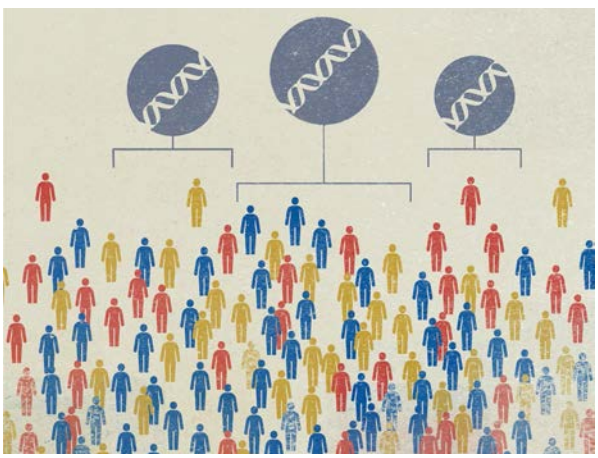
In the other study, Yazar et al. investigated how genetic variation influences regulation of the immune system across the general population, using scRNA-seq data from 1.27 million PBMCs collected from 982 healthy individuals of Northern European ancestry (the OneK1K cohort). The data were used to classify each cell into one of 14 transcriptionally distinct populations of immune cells. In each of these 14 cell types, Yazar et al. then used single-cell eQTL analysis to investigate how common genetic variants influence gene expression in a cell type-specific manner.

In total, they identified 26,597 independent *cis*-eQTLs and 990 *trans*-eQTLs, most of which were shown to have an allelic effect on gene expression that is cell-type specific. The eQTL findings were verified in two independent cohorts of individuals of European ($n = 113$) and Asian ($n = 89$) ancestry. "We also identified specific genetic variants that had a dynamic effect on gene expression as cells transition from one cell state to another — mainly B cells transitioning from naive to memory states" highlights corresponding author Joseph Powell.

Yazar et al. also performed Mendelian randomization and co-localization analyses of the eQTLs and genetic variants associated with seven common autoimmune diseases (including SLE and rheumatoid arthritis). They identified 305 loci as having cell type-specific causal effects, providing insight into how disease risk loci exert their effects, which is often through a single cell type.

Together, the studies provide valuable insights into inter-individual differences in immune function at the cellular level, and highlight the importance of cell type and context in mediating the effects of genetic associations.

Sarah Onuora



Credit: Alex Whitworth /Springer Nature Limited

ORIGINAL ARTICLES Perez, R. K. et al. Single-cell RNA-seq reveals cell type–specific molecular and genetic associations to lupus. *Science* https://doi.org/10.1126/science.abf1970 (2022) | Yazar, S. et al. Single-cell eQTL mapping identifies cell type–specific genetic control of autoimmune disease. *Science* https://doi.org/10.1126/science.abf3041 (2022)

RHEUMATOID ARTHRITIS

Targeting UHRF1 in RA

Epigenetic regulation has been proposed as a potential therapeutic strategy for the treatment of rheumatoid arthritis (RA), but a better understanding of the underlying mechanisms is needed for the development of novel therapeutics and/or biomarkers of the disease. New research demonstrates that the epigenetic regulator E3 ubiquitin-protein ligase UHRF1 suppresses arthritis pathogenesis in a mouse model and could provide a new avenue for RA therapy that does not directly affect the immune system.

The researchers first demonstrated that *Uhrf1* expression is markedly upregulated in mouse models of arthritis, namely the collagen antibody-induced arthritis (CAIA) and serum transfer-induced arthritis (STA) models, as well as in synovial tissue from patients with RA.

Uhrf1 expression in mouse joint tissue was found primarily in synovial fibroblasts. Moreover, depletion of *Uhrf1* specifically in synovial fibroblasts exacerbated experimental arthritis in CAIA and STA mice, which had more severe synovial hyperplasia and joint destruction than littermate controls, whereas synovial macrophage-specific depletion produced no notable phenotypes.

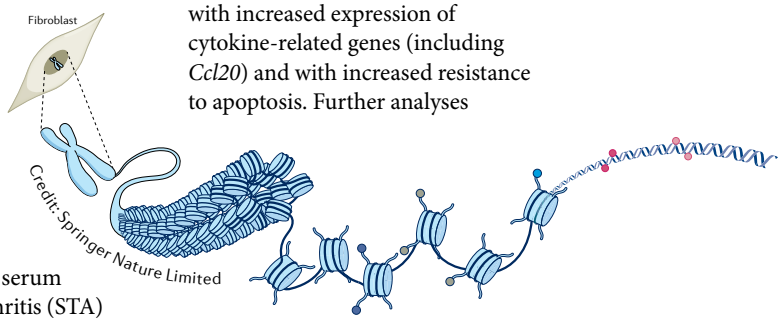
In mouse synovial fibroblasts, *Uhrf1* depletion was associated with increased expression of cytokine-related genes (including *Ccl20*) and with increased resistance to apoptosis. Further analyses indicated that *Uhrf1* influences mRNA expression of RA-related factors via modulation of DNA methylation.

> preserving expression of UHRF1 could suppress RA pathogenesis

In synovial tissue from patients with RA, expression of *UHRF1* positively correlated with response to DMARDs and negatively correlated with disease activity and accumulation of T helper 17 cells. By contrast, *UHRF1* knockdown was associated with increased expression of CCL20 and resistance to apoptosis.

Together, the findings suggested that preserving expression of UHRF1 could suppress RA pathogenesis. Consistent with this idea, stabilization of UHRF1 by administration of ryuvidine ameliorated features of arthritis in mouse models and inhibited hyperplasia in synovial fibroblast organoid cultures. The researchers suggest targeting UHRF1 could present a new option for treating RA, particularly for patients who do not respond to existing therapies.

Sarah Onuora



ORIGINAL ARTICLE Saeki, N. et al. Epigenetic regulator UHRF1 orchestrates pro-inflammatory gene expression in rheumatoid arthritis in a suppressive manner. *J. Clin. Invest.* https://doi.org/10.1172/JCI150533 (2022)

INFLAMMATION

Skin inflammation precedes lesions in cutaneous lupus erythematosus

Cutaneous lupus erythematosus (CLE) is common in systemic lupus erythematosus (SLE), and CLE skin lesions often remain even when systemic disease is successfully treated. Development of CLE lesions is thought to be associated with production of type I interferon in nonlesional skin, and research has now characterized the cellular relationships underlying this process.

In the new research, paired tissue samples from lesional and nonlesional skin of seven patients with CLE (six with diagnoses of SLE) were analysed by single-cell RNA sequencing (scRNA-seq). Skin samples from 14 healthy individuals were the controls. Data from 46,540 cells identified 26 distinct cell clusters and 10 major cell types.

> keratinocytes with the highest interferon-response scores were from nonlesional CLE samples

Keratinocytes comprised 55% of the sampled cells, and among 14 keratinocyte subclusters, four consisted of cells disproportionately derived from patients with CLE. Comparison of scRNA-seq data with previously determined cytokine-response signatures indicated that these CLE-enriched keratinocytes had expression patterns characteristic of response to interferons (particularly IFNα). Notably, the keratinocytes with the highest interferon-response scores were from nonlesional CLE samples. "This suggests that the predisposition for inflammation starts in the 'normal'-looking skin," says corresponding author Michelle Kahlenberg.

Credit: DrPixel/Getty Images

Analyses of fibroblasts and T cells identified one subcluster of each that was primarily from CLE samples, and that demonstrated interferon-response patterns. Among myeloid cells, T cell-stimulatory, interferon-secreting CD16$^+$ dendritic cells (DCs) were overrepresented in CLE samples versus controls.

Cell–cell communication was analysed via ligand–receptor pairs. In nonlesional CLE skin, the most interactive cell types were CD16$^+$ DCs and interferon-responsive fibroblasts. Analysis of changing expression patterns suggested that keratinocyte-derived type I interferon promotes activation of DCs that accumulate in skin of patients with CLE. "The keratinocytes turn the skin into an 'interferon bath' that endows certain immune cells that are recruited there with powerful pro-inflammatory capabilities," adds first author Allison Billi. The challenge now is to use these data for development of treatments for CLE.

Robert Phillips

ORIGINAL ARTICLE Billi, A. C. et al. Nonlesional lupus skin contributes to inflammatory education of myeloid cells and primes for cutaneous inflammation. *Sci. Transl Med.* **14**, eabn2263 (2022)

SYSTEMIC LUPUS ERYTHEMATOSUS

Novel *TLR7* variant causes lupus

Systemic lupus erythematosus (SLE) is a complex, multisystem autoimmune disease that has no single effective disease-modifying treatment owing to its heterogeneous and polygenic mechanisms of origin. Now, Brown et al. have identified a novel gene variant that causes SLE in humans, revealing a single pathway that could be targeted in patients with lupus.

To search for novel disease-causing variants, the authors sequenced the genome of a Spanish child with severe SLE. Their analysis identified a de novo and previously undescribed missense mutation (Y264H) in the gene encoding Toll-like receptor 7 (TLR7), a pathogen sensor located in many immune cells, including B cells. Sequencing analysis of several additional patients with SLE also revealed mutations in the *TLR7* gene, and there were previous suggestions that *TLR7* could be pathogenically relevant in SLE.

To determine whether $TLR7^{Y264H}$ causes SLE, the authors used CRISPR–Cas9 to introduce the orthologous variant into mice. Mice carrying the $TLR7^{Y264H}$ mutation went on to develop lupus symptoms, including an enlarged spleen, low platelet count and the presence of self-reactive antibodies. Moreover, histological analysis revealed evidence of damage to multiple tissues, as seen in human SLE.

> The discovery of this rare gene variant as a cause of SLE could lead to novel therapeutics

TLR7 activates the innate immune system in response to viral RNA and guanosine. Analysis of the functional consequences of $TLR7^{Y264H}$ revealed an increased affinity for endogenous guanosine, leading to TLR7 overactivation and the aberrant survival of B cells that bind to self-antigens. Strikingly, loss of MyD88 — an adaptor downstream of TLR7 — rescued the phenotype of $TLR7^{Y264H}$-carrying mice, confirming the importance of the TLR7 pathway in SLE.

The discovery of this rare gene variant as a cause of SLE could lead to novel therapeutics. As corresponding author Carola Vinuesa explains: "Targeting TLR7 may prove a more precise and effective therapy for treating lupus than current less specific approaches, and might have less side effects." Further work will focus on using the mouse model of human SLE to better understand lupus pathogenesis and test potential treatments.

Credit: Alex Whitworth/Springer Nature Limited

Michael Attwaters

ORIGINAL ARTICLE Brown, G. J. et al. *TLR7* gain-of-function genetic variation causes human lupus. *Nature* https://doi.org/10.1038/s41586-022-04642-z (2022)

BIOMARKERS

Toward pre-treatment prediction of biologic DMARD response in RA

In rheumatoid arthritis (RA), in the absence of precision medicine, many patients do not respond to biologic DMARDs (bDMARDs). The latest results from the R4RA randomized clinical trial comparing rituximab and tocilizumab therapies in patients with RA demonstrate that analysis of tissue samples enables prediction of drug response prior to treatment.

RA is a heterogeneous disease, and the mechanisms that underlie an individual's treatment response are not yet known. RA-associated expression of bDMARD targets varies, and previous results from the R4RA trial showed that response to rituximab is affected by a synovial deficiency in $CD20^+$ B cells that occurs in ~50% of patients with RA.

R4RA investigators have now conducted detailed histological and molecular analyses of biopsy-derived synovial tissue samples from 164 patients with RA who were randomly assigned to rituximab or tocilizumab treatment. At 16 weeks, nonresponders were switched to the alternative treatment. All participants received, but did not adequately respond to, conventional synthetic DMARDs and anti-TNF bDMARDs prior to R4RA enrolment.

Analysis of cell types and differentially expressed genes in baseline samples of responders and nonresponders indicated that rituximab response was associated with a prevalence of lymphoid cells, whereas tocilizumab response was associated with the additional presence of myeloid cells. Lack of response to either bDMARD was associated with predominance of stromal cells, with few immune cells. Further analysis of the stromal cells identified Dickkopf 3-expressing fibroblasts as potential targets for overcoming DMARD resistance.

> analysis of tissue samples enables prediction of drug response prior to treatment

Credit: Alex Whitworth/Springer Nature Limited

Machine learning was applied to the results of the molecular studies, to generate models for prediction of bDMARD response or refractoriness. The optimal model for prediction of rituximab response included 40 genes and had an area under the curve (AUC) of 0.744, whereas that for tocilizumab response involved 39 genes (AUC = 0.681), and a 53-gene model (AUC = 0.686) was optimal for prediction of a refractory state. Further studies should improve upon these predictive values, and provide additional support for adoption of the routine use of synovial biopsy to establish a precision-medicine approach to the treatment of RA.

Robert Phillips

ORIGINAL ARTICLE Rivellese, F. et al. Rituximab versus tocilizumab in rheumatoid arthritis: synovial biopsy-based biomarker analysis of the phase 4 R4RA randomized trial. *Nat. Med.* https://doi.org/10.1038/s41591-022-01789-0 (2022)

RHEUMATOID ARTHRITIS

Towards the patient-centred care of rheumatoid arthritis

Zara Izadi and Gabriela Schmajuk

New research reveals that outcomes for patients with rheumatoid arthritis are affected less by pre-existing comorbid conditions than by socioeconomic factors. Future research should address the mechanisms of this relationship and develop holistic treatment approaches that reduce disparities attributed to socioeconomic status.

Refers to Busby, A. D. et al. The role of comorbidities alongside patient and disease characteristics on long-term disease activity in RA using UK inception cohort data. *Rheumatology* https://doi.org/10.1093/rheumatology/keac139 (2022).

To be truly patient centred, the management of rheumatoid arthritis (RA) requires consideration of comorbidities. In 2016, EULAR implemented a new initiative with the intention of improving the reporting and prevention of comorbidities in chronic inflammatory rheumatic diseases[1]. In 2021, the ACR's RA treatment guidelines were updated such that recommendations were no longer stratified by early versus late RA disease duration, but rather according to the presence of comorbidities, current disease activity and prior therapies, because these factors were considered to be more relevant than disease duration for most treatment decisions. However, newly published research suggests that comorbidities have less association with RA disease activity than some sociodemographic and clinical factors[2].

Compared with the general population, individuals with RA have a higher prevalence of comorbidities such as chronic pulmonary disease, chronic kidney disease, heart disease, hypertension, diabetes and malignancies[3]. Comorbidities can influence RA disease activity through various pathways. First, certain comorbidities might preclude treatment escalation to biologic DMARDs or targeted synthetic DMARDs. Second, comorbidities can influence patient global assessment and/or biomarkers of inflammation, resulting in overestimation of composite RA disease-activity measures. Third, the relationship between comorbidities and RA outcomes is confounded and modified by multiple factors, including patient demographics, socioeconomic status (SES) and lifestyle factors such as exercise, diet, sleep and smoking[4–6] (FIG. 1).

In an article published in *Rheumatology*, Busby et al. have presented a timely addition to the research on comorbidities in people with RA, exploring relationships between comorbidities, sociodemographics and RA clinical characteristics at baseline, and RA disease activity 5 years and 10 years after diagnosis[2]. The researchers analysed data from 2,701 individuals with RA recruited into two multicentre, prospective cohorts in the UK. Comorbidities were measured using the Rheumatic Disease Comorbidity Index (RDCI), on a continuous scale ranging from zero to nine. Disease activity was assessed using the 28-joint Disease Activity Score (DAS28), with scores dichotomized into 'remission or low' (<3.2) and 'moderate or high' (≥3.2) disease activity. The Index of Multiple Deprivation (IMD), derived from patient postcodes, was used as a measure of SES.

The results of the study showed that RDCI assessed at baseline was not associated with disease activity at 5 years or 10 years in unadjusted or adjusted analyses[2]. Although a few individual comorbid conditions were associated with disease activity in bivariate analyses, these associations did not persist in adjusted analyses. Lower SES, female gender and worse functional status were associated with higher disease activity in unadjusted and adjusted analyses at both time points. In adjusted analyses, seropositivity and presence of erosions were also associated with higher disease activity at 5 years and 10 years, respectively. Interestingly, age at RA diagnosis was not associated with disease activity at either time

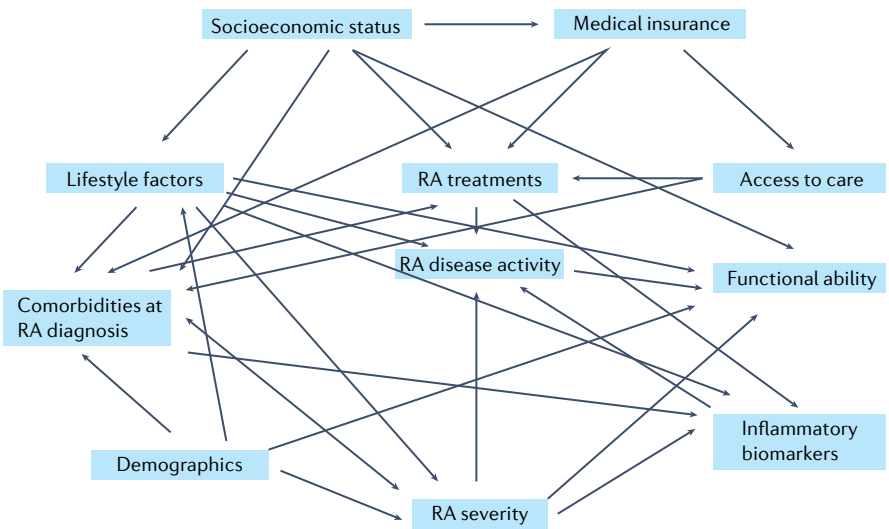


Fig. 1 | **Directed acyclic graph showing complex relationships between comorbidities, clinical and patient-reported outcomes and patient sociodemographics in rheumatoid arthritis.** Demographics include age, sex and race and/or ethnicity. Socioeconomic status includes education and income. Lifestyle factors include exercise, diet, sleep and smoking. Inflammatory biomarkers include erythrocyte sedimentation rate and C-reactive protein. RA, rheumatoid arthritis.

point. Prior studies conducted in the UK have shown that people living in areas with more neighbourhood deprivation have higher rates of premature mortality, morbidity and worse health outcomes[7]. The results of Busby et al. add to prior research by highlighting SES as an important determinant of long-term RA disease activity independent from pre-existing comorbid conditions[2]. This finding provides a powerful motivation for further research that seeks to unravel the mechanisms of the effect of SES on health outcomes in people with RA.

> Lower SES, female gender and worse functional status were associated with higher disease activity

Further confirmatory longitudinal studies will be necessary to investigate the causal relationship between pre-existing comorbidities and RA disease trajectory while accounting for treatment patterns as well as other clinical and sociodemographic factors. A considerable proportion of patients recruited into the study of Busby et al. were lost to follow-up (36.4% at 5 years and 69.6% at 10 years), and the 185 patients (6.8%) who died during the study period were excluded from analyses[2]. Patients who remained in the study were younger, were less likely to smoke and had fewer comorbidities (including heart disease, depression and cancer) than those who were lost to follow-up. Patients who were observed at 10 years also had higher SES and fewer erosions at diagnosis compared with those who were lost to follow-up. These limitations may be addressed in further analyses in cohorts with more complete follow-up. Alternatively, attrition bias arising from loss to follow-up may be quantified in future work by the use of sensitivity analyses or weighting techniques. Analysing comorbidities individually or grouped into clinically meaningful categories will aid interpretability and make the exposure less ambiguous, which is a key requirement for establishing causal inference[8]. Comorbidities that are not included in the RDCI but that influence RA outcomes or are contraindicated with certain RA medications, such as fibromyalgia, infections and kidney or liver disease, also need to be incorporated into further analyses.

Early diagnosis and treatment improves outcomes and is an overarching principle in the management of RA[9]. Although access to care is vital for timely diagnosis and treatment, the results presented by Busby et al. suggest that utilization of rheumatology care is not sufficient to eliminate disparate health outcomes for individuals with RA[2]. In results published in 2021, we similarly demonstrated that, even among people with RA who have access to rheumatologist care in the USA, physical function is worse in groups with lower SES and rates of decline are faster, compared with patients with higher SES[6]. Improving disparities in RA outcomes will require an understanding of the reasons for higher disease activity among patients in the lower SES groups. Hypotheses include that these disparities may result from delays in initial diagnosis and treatment or from either disparate treatment or factors that impede adequate treatment, such as depression, low health literacy and lower adherence to treatments than in individuals with higher SES. Medication adherence can be affected by costs or patients' trust in their clinicians or the health-care system[10].

Strategies to incorporate social determinants of health such as safety, nutrition, housing, social connectedness and positive emotions into the management of RA have the potential to complement and enhance outcomes achievable with treat-to-target using pharmacological agents. A holistic treatment plan should involve multidisciplinary care teams. Tailoring the management plan to the cultural and linguistic needs of the patient is also important to address these fundamental issues. The results of Busby et al.[2] reinforce the importance of this holistic approach to care and highlight SES as a key consideration when risk-stratifying and managing individuals with RA.

Zara Izadi[1] ✉ *and Gabriela Schmajuk*[1,2]

[1]*School of Medicine, Division of Rheumatology, University of California San Francisco, San Francisco, CA, USA.*

[2]*San Francisco VA Medical Center, San Francisco, CA, USA.*

✉*e-mail: Zara.Izadi@ucsf.edu*

https://doi.org/10.1038/s41584-022-00794-4

1. Baillet, A. et al. Points to consider for reporting, screening for and preventing selected comorbidities in chronic inflammatory rheumatic diseases in daily practice: a EULAR initiative. *Ann. Rheum. Dis.* **75**, 965–973 (2016).
2. Busby, A. D. et al. The role of comorbidities alongside patient and disease characteristics on long-term disease activity in RA using UK inception cohort data. *Rheumatology* https://doi.org/10.1093/rheumatology/keac139 (2022).
3. Mikuls, T. R. Co-morbidity in rheumatoid arthritis. *Best Pract. Res. Clin. Rheumatol.* **17**, 729–752 (2003).
4. Zaccardelli, A., Friedlander, H. M., Ford, J. A. & Sparks, J. A. Potential of lifestyle changes for reducing the risk of developing rheumatoid arthritis: is an ounce of prevention worth a pound of cure? *Clin. Ther.* **41**, 1323–1345 (2019).
5. Barton, J. L. et al. Racial and ethnic disparities in disease activity and function among persons with rheumatoid arthritis from university-affiliated clinics. *Arthritis Care Res.* **63**, 1238–1246 (2011).
6. Izadi, Z. et al. Socioeconomic disparities in functional status in a national sample of patients with rheumatoid arthritis. *JAMA Netw. Open* **4**, e2119400 (2021).
7. Lewer, D. et al. Premature mortality attributable to socioeconomic inequality in England between 2003 and 2018: an observational study. *Lancet Public Health* **5**, e33–e41 (2020).
8. Pearl, J. On the consistency rule in causal inference: axiom, definition, assumption, or theorem? *Epidemiology* **21**, 872–875 (2010).
9. Burgers, L. E., Raza, K. & van der Helm-van Mil, A. H. Window of opportunity in rheumatoid arthritis-definitions and supporting evidence: from old to new perspectives. *RMD Open* **5**, e000870 (2019).
10. Harrold, L. R. et al. Cost-related medication nonadherence in older patients with rheumatoid arthritis. *J. Rheumatol.* **40**, 137–143 (2013).

Acknowledgements
G.S. is supported by the Russell/Engleman Medical Research Center for Arthritis.

Competing interests
The authors declare no competing interests.

PAEDIATRIC RHEUMATOLOGY

2021 ACR guideline for JIA reflects changes in practice

Roberta Naddei and Angelo Ravelli

The new ACR guideline for the treatment of juvenile idiopathic arthritis provides an update on several important topics, including management of oligoarthritis, temporomandibular joint arthritis and systemic-onset arthritis. Overall, the new guideline reflects changes in practice, but also highlights a concerning lack of high-quality evidence.

Refers to Onel, K. B. et al. 2021 American College of Rheumatology guideline for the treatment of juvenile idiopathic arthritis: therapeutic approaches for oligoarthritis, temporomandibular joint arthritis, and systemic juvenile idiopathic arthritis. *Arthritis Rheumatol.* **74**, 553–569 (2022).

The ACR has updated their guidelines for the treatment of juvenile idiopathic arthritis (JIA) to reflect the therapeutic advances, improved understanding of disease pathogenesis and changes in practice that have occurred since previous ACR recommendations were published in 2011 (REF.[1]) and 2013 (REF.[2]). Considering the breadth of the topic, the decision was made to separate the new guidelines into several parts. Herein, we discuss the guideline proposing therapeutic approaches to oligoarthritis, temporomandibular joint (TMJ) arthritis and systemic-onset JIA (sJIA) with and without macrophage activation syndrome (MAS)[3]. Notably, the new recommendations for oligoarthritis differ in several important respects from previous guidance (TABLE 1); moreover, management of TMJ arthritis is a new addition to the guidelines and the sJIA guidance reflects the current prominence of anti-cytokine therapy.

The 2021 guideline conditionally recommends a trial of NSAIDs administered according to a dosing schedule (rather than on-demand) as part of the initial therapy for active oligoarthritis, active TMJ arthritis and sJIA without MAS. However, owing to the potential adverse effects of NSAIDs and their limited efficacy, it was determined that such a trial should be brief. The panellists did not reach an agreement about the appropriate duration of treatment, and some preferred that the administration of NSAIDs be avoided altogether.

Even though the evidence supporting their use was of low quality, intraarticular glucocorticoids (IAGCs) are strongly recommended as part of the initial therapy for oligoarthritis, owing to a low risk of adverse effects and a high likelihood of sustained response. Triamcinolone hexacetonide is the favoured IAGC preparation because its effectiveness is more durable than that of triamcinolone acetonide. Unlike the 2011 recommendations[1], no advice is provided regarding the expected duration of effect or the interval between repeat IAGC injections.

Methotrexate is recommended as the preferred conventional synthetic DMARD (csDMARD) for oligoarthritis in the event of inadequate response to NSAIDs and/or IAGCs. This sequence represents a diversion from 2011 recommendations[1], which allowed for the introduction of methotrexate as a first-line treatment for patients with a high level of disease activity and features of poor prognosis.

Although biologic DMARDs (bDMARDs) are strongly recommended in case of incomplete response to (or intolerance of) NSAIDs and/or IAGCs and at least one csDMARD, unlike the 2011 recommendations[1] no particular bDMARD is preferred. This decision was attributed to the demonstration that bDMARDs other than TNF inhibitors can be efficacious for oligoarthritis and to the notion that head-to-head trials of bDMARDs are lacking.

The 2021 guideline for the management of oligoarthritis also includes a conditional recommendation to consider risk factors for poor outcome to guide therapeutic decisions, including rapid escalation of treatment or choice of an alternative medication. The presence of features of poor prognosis was also a driver of treatment intensification in the 2011 recommendations[1].

The optimal treatment for active TMJ arthritis was not specifically addressed in previous recommendations and remains controversial. The main uncertainty regards the indication for IAGCs, which, although potentially effective in alleviating joint symptoms and restoring function, can lead to serious TMJ-specific adverse events, especially heterotopic ossification and impaired mandibular growth[4]. For this reason, the panellists who developed the 2021 ACR guideline concluded, prudently, that IACGs should be used sparingly for children with active TMJ arthritis, preferably those who are skeletally mature.

Considering the distinctive vulnerability of the TMJ to structural damage and the effect of TMJ involvement on activities of daily living, the 2021 guideline encourages the early introduction of csDMARDs, with methotrexate being the preferred agent. Despite limited evidence, bDMARDs are recommended in case of failure of NSAIDs and/or IAGCs and at least one csDMARD. No particular bDMARD is preferred, although TNF inhibitors are recognized as the most commonly used.

Oral glucocorticoids are conditionally recommended against as initial therapy for both active oligoarthritis and TMJ arthritis. Recognizing that these medications might be administered to rapidly alleviate severe symptoms when IAGCs are not available or feasible, or to act as a bridge therapy until the onset of action of DMARDs, the guideline recommends that treatment with oral glucocorticoids should be limited to the lowest effective dose for the shortest duration possible. The guideline also conditionally recommends against the use of oral glucocorticoids as initial monotherapy for patients with sJIA without MAS, while acknowledging that these medications might help control systemic and joint manifestations until bDMARDs can be started.

For sJIA, the guideline separately addresses the management of this condition in the absence or presence of MAS. The main change from previous guidelines concerns the use of IL-1 and IL-6 inhibitors as initial monotherapy for all instances of sJIA without MAS. This recommendation was conditionally made in light of the favourable outcomes obtained with administration of the IL-1 receptor antagonist anakinra as first-line monotherapy in patients with new-onset sJIA[5] and the ability of IL-1 and IL-6 inhibitors to enable glucocorticoid tapering[6,7]. Although some panellists expressed a preference for starting treatment with the short-acting agent anakinra, in the absence of head-to-head comparisons with IL-6 inhibitors or other IL-1 blockers no one agent was endorsed.

csDMARDs are strongly recommended against as initial monotherapy for sJIA without MAS, given their documented lack of efficacy in controlling systemic features; however, the combination of csDMARDs with bDMARDs

Table 1 | **Comparison of 2021 and 2011 ACR recommendations for treatment of oligoarthritis**

Clinical scenario	2011 ACR recommendations[1]	2021 ACR guideline[3]
First-line therapy	NSAIDs recommended (maximum duration 2 months)	NSAIDs conditionally recommended (duration not specified)
	IAGCs recommended (THA preferred)	IAGCs strongly recommended (THA strongly preferred)
	Methotrexate recommended for patients with high disease activity and features of poor prognosis	Oral glucocorticoids conditionally recommended against
Inadequate response to first-line therapy	Methotrexate recommended	csDMARDs strongly recommended (methotrexate conditionally preferred)
Inadequate response to csDMARDs	TNF inhibitors recommended	bDMARDs strongly recommended (no preferred agent)

bDMARD, biologic DMARD; csDMARD, conventional synthetic DMARD; IAGC, intraarticular glucocorticoid; THA, triamcinolone hexacetonide.

is an option for children with severe arthritis. Options for children with residual arthritis despite anti-IL-1 or anti-IL-6 therapy included the addition of methotrexate or switching to another bDMARDs, such as a TNF inhibitor or abatacept.

For patients with sJIA and MAS, IL-1 and IL-6 inhibitors were deemed superior to calcineurin inhibitors for achieving a state of inactive disease and resolving MAS. The guideline acknowledges, however, that monotherapy with these agents might not be sufficient for the patients who are most severely ill; in these patients, the combination of bDMARDs with glucocorticoids and calcineurin inhibitors might be necessary.

The guideline emphasizes the importance of tapering and discontinuing glucocorticoids once inactive disease has been attained in patients with sJIA (with or without a history of MAS) receiving these medications in combination with DMARDs, owing to the harms related to long-term administration of glucocorticoids. Although discontinuation of DMARDs after attainment of inactive disease is also recommended, the guideline underscores the current lack of clarity regarding the timeline and method of tapering. Patients and caregivers who were involved in developing the guideline tended to prefer increasing the interval between doses over decreasing the dosage.

Overall, the 2021 ACR guideline provides valuable and up-to-date advice on how to manage JIA in clinical practice. The new recommendations incorporate modern therapeutic goals, which include early achievement of disease control, sparing use of glucocorticoids, prevention of disease-related and treatment-related morbidity and the rational withdrawal of treatment after attainment of an inactive disease state. Additional points of merit are the participation of a patient and caregiver panel in developing the new guideline and the focus on shared decision-making in its guiding principles.

A matter of concern, however, is that most of the evidence on which the guideline is based is of low quality. This weakness underscores the need for further studies in order to formulate more robust recommendations, especially head-to-head trials that define the optimal sequence and roles of csDMARDs and bDMARDs. Future investigations should also aim to devise individualized approaches to treatment that enable the tailoring of therapeutic interventions to a particular child and abbreviate the time to achieve disease remission. The research agenda should also include trials comparing different approaches to dose reduction and tapering, which are of crucial relevance to the optimal long-term management of JIA.

Roberta Naddei[1,2] *and Angelo Ravelli*[3,4]✉

[1]*Dipartimento di Scienze Mediche Traslazionali, Sezione di Pediatria, Università degli Studi di Napoli Federico II, Naples, Italy.*

[2]*Clinica Pediatrica e Reumatologia, IRCCS Istituto Giannina Gaslini, Genoa, Italy.*

[3]*Direzione Scientifica, IRCCS Istituto Giannina Gaslini, Genoa, Italy.*

[4]*Dipartimento di Neuroscienze, Riabilitazione, Oftalmologia, Genetica e Scienze Materno-Infantili (DINOGMI), Università degli Studi di Genova, Genoa, Italy.*

✉*e-mail: angeloravelli@gaslini.org*

https://doi.org/10.1038/s41584-022-00787-3

1. Beukelman, T. et al. 2011 American College of Rheumatology recommendations for the treatment of juvenile idiopathic arthritis: initiation and safety monitoring of therapeutic agents for the treatment of arthritis and systemic features. *Arthritis Care Res. (Hoboken)* **63**, 465–482 (2011).
2. Ringold, S. et al. 2013 update of the 2011 American College of Rheumatology recommendations for the treatment of juvenile idiopathic arthritis: recommendations for the medical therapy of children with systemic juvenile idiopathic arthritis and tuberculosis screening among children receiving biologic medications. *Arthritis Rheum.* **65**, 2499–2512 (2013).
3. Onel, K. B. et al. 2021 American College of Rheumatology guideline for the treatment of juvenile idiopathic arthritis: therapeutic approaches for oligoarthritis, temporomandibular joint arthritis, and systemic juvenile idiopathic arthritis. *Arthritis Rheumatol.* **74**, 553–569 (2022).
4. Stoll, M. L. et al. Risk factors for intraarticular heterotopic bone formation in the temporomandibular joint in juvenile idiopathic arthritis. *J. Rheumatol.* **45**, 1301–1307 (2018).
5. Ter Haar, N. M. et al. Treatment to Target using recombinant interleukin-1 receptor antagonist as first-line monotherapy in new-onset systemic juvenile idiopathic arthritis: results from a five-year follow-up study. *Arthritis Rheumatol.* **71**, 1163–1173 (2019).
6. Ruperto, N. et al. Two randomized trials of canakinumab in systemic juvenile idiopathic arthritis. *N. Engl. J. Med.* **367**, 2396–2406 (2012).
7. De Benedetti, F. et al. Randomized trial of tocilizumab in systemic juvenile idiopathic arthritis. *N. Engl. J. Med.* **367**, 2385–2395 (2012).

Competing interests

A.R. declares that he received honoraria and research grants from Abbvie, Alexion, Angelini, BMS, Novartis, Pfizer, Reckitt-Benkiser, Roche and SOBI. R.N. declares no competing interests.

REVIEWS

From risk to chronicity: evolution of autoreactive B cell and antibody responses in rheumatoid arthritis

Hans Ulrich Scherer, Diane van der Woude and Rene E. M. Toes ✉

Abstract | The presence of disease-specific autoantibody responses and the efficacy of B cell-targeting therapies in rheumatoid arthritis (RA) indicate a pivotal role for B cells in disease pathogenesis. Important advances have shaped our understanding of the involvement of autoantibodies and autoreactive B cells in the disease process. In RA, autoantibodies target antigens with a variety of post-translational modifications such as carbamylation, acetylation and citrullination. B cell responses against citrullinated antigens generate anti-citrullinated protein antibodies (ACPAs), which are themselves modified in the variable domains by abundant N-linked glycans. Insights into the induction of autoreactive B cells against antigens with post-translational modifications and the development of autoantibody features such as isotype usage, epitope recognition, avidity and glycosylation reveal their relationship to particular RA risk factors and clinical phenotypes. Glycosylation of the ACPA variable domain, for example, seems to predict RA onset in ACPA$^+$ healthy individuals, possibly because it affects B cell receptor signalling. Moreover, ACPA-expressing B cells show dynamic phenotypic changes and develop a continuously proliferative and activated phenotype that can persist in patients who are in drug-induced clinical remission. Together, these findings can be integrated into a conceptual framework of immunological autoreactivity in RA, delineating how it develops and persists and why disease activity recurs when therapy is tapered or stopped.

Rheumatoid arthritis (RA) is a chronic autoimmune disease that causes inflammation of synovial joints, as well as other systemic effects in multiple organs. Although disease activity can often be controlled by anti-rheumatic medication, most patients require life-long treatment because current therapeutic strategies alleviate signs and symptoms but do not cure the disease. Half of patients with RA harbour autoantibodies at first presentation[1]. Because autoantibody-positive patients rarely achieve long-term drug-free remission, their proportion increases in patient populations as the length of follow-up increases[2].

The first autoantibody identified in RA was rheumatoid factor (RF)[3]. Results showed that serum from patients with RA could enhance agglutination of red blood cells induced by rabbit serum that contained anti-red blood cell antibodies. Not all patients with RA had serum with this property, however, and also few individuals without RA had serum that contained this 'agglutination-activating factor'. Today, it is well established that this factor, RF, is not present in all patients with RA, nor is it completely specific to RA. RFs are antibodies that recognize the 'fragment crystallizable' (Fc) region of IgG when the IgG is complexed with antigen[4]. The capacity of RF to bind IgG and form immune complexes may have evolved originally as a rapid-response antigen-clearance mechanism, particularly for highly ordered, repetitive antigens[5,6]. Consistently, the mutational and transcriptional profile of RF-expressing memory B cells indicates a particular responsiveness to innate immune stimuli[7]. In the context of RA, RF is considered to diverge from this presumably protective function and to enhance inflammatory responses, as both isotype use and epitope-recognition patterns of RF in RA seem to be distinct from those of RF in other conditions[8,9]. Notably, RF and anti-citrullinated protein antibodies (ACPAs) frequently co-occur, and although RF may amplify immune-cell activation, RF positivity is not unique to RA. In fact, many associations of RF with RA disease features and phenotypes seem to be driven by the concomitant presence of ACPAs rather than RF itself[10]. For this reason, and for reasons of focus and constraints of space, we refrain here from expanding on the RF B cell response in detail, and focus on ACPAs

Department of Rheumatology, Leiden University Medical Center, Leiden, The Netherlands.

✉*e-mail: r.e.m.toes@lumc.nl*

https://doi.org/10.1038/s41584-022-00786-4

Key points

- Anti-modified-protein antibodies exhibit broad cross-reactivity towards multiple post-translational modifications.
- Compared with other antibodies, anti-citrullinated protein antibodies (ACPAs) are characterized by the presence of high numbers of N-linked glycans in the variable domain.
- Generation of the amino acid sequences that are required for N-linked glycosylation is mediated by somatic hypermutation and associated with the presence of *HLA*-shared epitope alleles.
- Generation of glycosylation sites occurs prior to the development of rheumatoid arthritis and likely provides a selective advantage to ACPA-expressing B cells.
- ACPA-expressing B cells, unlike B cells directed against recall antigens, display an activated and proliferative phenotype in rheumatoid arthritis, suggesting that they undergo continuous antigenic triggering.
- Even in patients in drug-induced clinical remission, ACPA-expressing B cells still display an activated, proliferative phenotype, indicating a lack of induction of immunological remission.

and the evolution of the B cell response to antigens with post-translational modifications (PTMs).

Along with RF, ACPAs are autoantibodies that are routinely assessed in the diagnosis of RA. In the history of their discovery, in 1964 'anti-perinuclear factors' were identified that were specifically found in patients with RA[11]. In the late 1990s, the amino acid citrulline (an enzymatic modification of arginine) was identified as an essential component of antigens recognized by anti-perinuclear factors[12,13]. This discovery resulted in a surge of novel insights into the biology and epidemiology of these autoantibodies, now known as ACPAs. Owing to their specificity, ACPAs are now part of the EULAR–ACR 2010 classification criteria for RA[14]. An important feature that emerged by studying reactivity to other PTMs is that ACPAs not only recognize a plethora of citrullinated proteins and peptides, but are also cross-reactive with proteins containing the modified amino acids homocitrulline and acetyllysine[15–20].

In this Review, we describe the relationship between ACPAs and related autoantibody responses in RA. We highlight recent insights into how autoreactive B cell responses to modified antigens (with an emphasis on checkpoints controlling B cell activation) could be induced, and discuss our current understanding of how the autoantibody response is shaped and how it correlates with disease (and pre-disease) stages and the two most prominent risk factors for RA (HLA and smoking). We also discuss the possible explanations and implications of the continuous and persistent activation of the underlying autoreactive B cell response.

Anti-modified-protein immune responses

ACPAs (similar to RF) can be present long before the onset of disease. In fact, ~1% of healthy individuals harbour these autoantibodies[21–26]. Notably, unlike in RA, their presence in healthy individuals is not necessarily stable, and seroconversion to the ACPA-negative state occurs with remarkable frequency (in up to ~70% of individuals), especially in the absence of RF[27,28]. By contrast, ACPA persistence, the concomitant presence and persistence of RF and, in particular, 'evolution' of the ACPA response are observed prior to the onset of disease in individuals who transition to RA[29,30]. This pre-disease evolution involves variation in isotype usage, serum ACPA concentration, variable (V)-domain glycosylation and the citrullinated-epitope recognition profile.

ACPAs, by definition, recognize citrullinated proteins. However, ACPAs can also cross-react with other PTMs, such as homocitrulline, an amino acid that resembles citrulline, but with addition of a methylene group (FIG. 1a). Unlike citrulline, which is formed by enzymatic conversion of arginine by peptidylarginine deiminases, homocitrulline is derived from a non-enzymatic modification of lysine after reaction with cyanate. The reaction of cyanate with amines is called carbamylation, and antibodies that recognize homocitrulline are named anti-carbamylated protein antibodies (ACarPAs)[31]. Likewise, ACPAs can also cross-react with proteins that contain acetyllysine residues[32], which are formed by the PTM of lysine by acetyltransferases such as histone acetyltransferase[33]. Although ACPAs can recognize both carbamylated and acetylated proteins, both modifications differ from citrulline in structure and in location within a protein, as they are derived from different amino acids. Therefore, ACPAs, ACarPAs and anti-acetylated-protein antibodies (AAPAs) are often seen as separate autoantibodies that are collectively summarized as anti-modified-protein antibodies (AMPAs)[31,34,35].

Although AMPAs can cross-react with citrulline, homocitrulline and acetyllysine modifications (FIG. 1), not all AMPAs display cross-reactivity to all modifications or do so to the same extent[18,19,35,36]. Variable cross-reactivity occurs between individual monoclonal antibodies and also within patient populations, as AMPA-positive patients can possess reactivity to one, two or all three PTMs[34,37]. Currently available evidence indicates that most AMPA-positive patients have reactivity to at least two of the PTMs, suggesting a commonality in the way in which these modifications interact with the antigen-binding sites of AMPAs. In mice, AMPA responses to more than one PTM can arise from exposure to antigens with only one type of modification, indicating that multiple AMPA responses can originate from a common B cell response[17]. Likewise, human B cells expressing ACPAs as B cell receptors (BCRs) can be activated by citrullinated, acetylated or carbamylated antigens[18] (FIG. 1b). Thus, the modified antigen that incites a particular AMPA response can differ from the modified antigen that enables detection of that AMPA. Consequently, a wealth of antigens could potentially activate AMPA-expressing B cells, both at the time of their initial priming in lymph nodes, and in peripheral tissues where they might encounter different modified antigens.

Despite evidence of the promiscuity of AMPAs, other results indicate that the antigenic backbone and flanking regions around a particular modification can affect reactivity, as not all AMPAs show cross-reactivity to all modified antigens[18,19,36]. The explanation for these contrasting observations is not yet known, but one suggestion is that ACPAs can be divided into 'promiscuous' and 'private' or 'specific' types[38]. This concept is based on results of molecular structural analyses of ACPAs of human and

murine origin[39,40]. Although further evidence is required, it is tempting to speculate that the reactivity patterns of ACPA, ACarPA and AAPA responses represent a sliding scale from antibodies that only require a PTM on the target antigen (promiscuous AMPAs) to antibodies that require one specific modification (for example, citrulline) as well as specific flanking regions for target binding (private or specific ACPAs)[19] (FIG. 1c).

The particulars of a 'specificity scale' might differ according to the patient and/or the disease stage, potentially explaining the variation in AMPA reactivity patterns and modified-epitope profiles. Notably, in addition to the AMPA BCR repertoire generated during the initial break of tolerance, the 'specificity scale' of individual AMPAs (and hence BCRs) might be influenced by the number of somatic mutations affecting the antibody V domain, as suggested by the results of studies in which such mutations were experimentally removed[36]. Hence, somatic hypermutation (SHM) could result in epitope spreading of the ACPA response as observed before disease onset in ACPA-positive individuals who transition to RA. However, SHM could also lead to loss of reactivity. Therefore, and as cross-reactivity can already be detected in germline-encoded clones of the IgM isotype, SHM might 'shape' rather than expand the AMPA repertoire[41].

Checkpoints for B cell activation

AMPA B cell responses are induced long before disease onset[22,23]. However, the routes leading to the initial activation of this autoimmune B cell response are not yet known. Immune tolerance normally prevents the development of detrimental lymphocyte responses to self-antigens. Several layers of control prohibit the emergence of unwanted and potentially harmful B cells such as those that generate AMPAs. In particular, receptor editing and clonal deletion in the bone marrow (central tolerance) contribute to tolerance in a polyclonal B cell population[42,43]. Developing, immature B cells in the bone-marrow environment are examined for reactivity to extracellular antigens, which limits self-reactivity of the mature B cell population in the spleen and lymph nodes. Receptor editing involves the generation of a 'revised' BCR with a 'harmless' specificity through secondary recombination affecting (mainly) the light chain of the antibody. Such secondary rearrangements are mediated by the action of enzymes encoded by *RAG1* and *RAG2*, which are also responsible for the primary rearrangement of the *V(D)J* gene segments. These processes are considered to be the main mechanism of tolerance induction, and they preserve cell numbers in the emerging B cell population[44] while minimizing the repertoire of self-reactive elements. However, prevention of autoreactivity and central tolerance in the bone marrow might also require clonal deletion or unresponsiveness to antigen (anergy), as reviewed elsewhere[45]. These general mechanisms are well characterized. Their relevance for the induction of autoreactive B cells in RA to PTM antigens needs further investigation.

Central tolerance is essential to shape the developing immature B cell population. Overly stringent central tolerance, however, might affect the breadth of the BCR repertoire, resulting in imbalance between the ability to respond to invasive agents and the development of autoreactivity. Clearly, central tolerance is not absolute, and potentially self-reactive B cells populate the peripheral immune system after escaping central-tolerance mechanisms. Therefore, peripheral checkpoints also act to prevent the emergence of B cell-mediated self-reactivity[46] (FIG. 2). Follicular dendritic cells (FDCs) are intimately involved in the induction of B cell responses through the presentation of antigen and the production of cytokines and other factors that attract, activate and promote the survival of B cells[47–49]. FDCs typically present

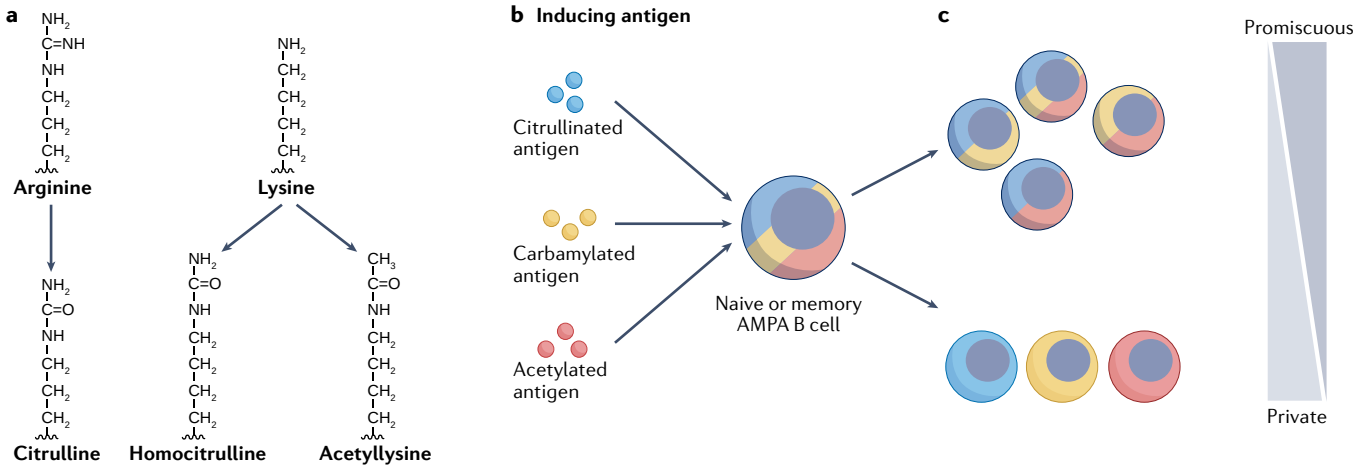


Fig. 1 | **Post-translational modifications and autoantibody cross-reactivity. a** | Anti-modified-protein antibodies (AMPAs) recognize a variety of post-translational modifications (PTMs) of amino acid residues, in particular, citrulline (a modification of arginine), and homocitrulline and acetyllysine (modifications of lysine). **b** | Because of their cross-reactivity, AMPA-expressing B cells can be stimulated by antigens with any of these PTMs. Cross-reactivity is a feature of both naive, IgM-expressing B cells and class-switched memory B cells. **c** | Depending on the stimulating post-translationally modified antigen and the initial affinity of the B cell receptor for the respective modification, PTM recognition in the context of co-stimulatory signals can stimulate the development of different AMPA repertoires, in which both 'private' and 'promiscuous' AMPAs can be found. Repetitive antigen recognition and somatic hypermutation likely modify and shape affinity for modified antigens, thereby creating a dynamic repertoire.

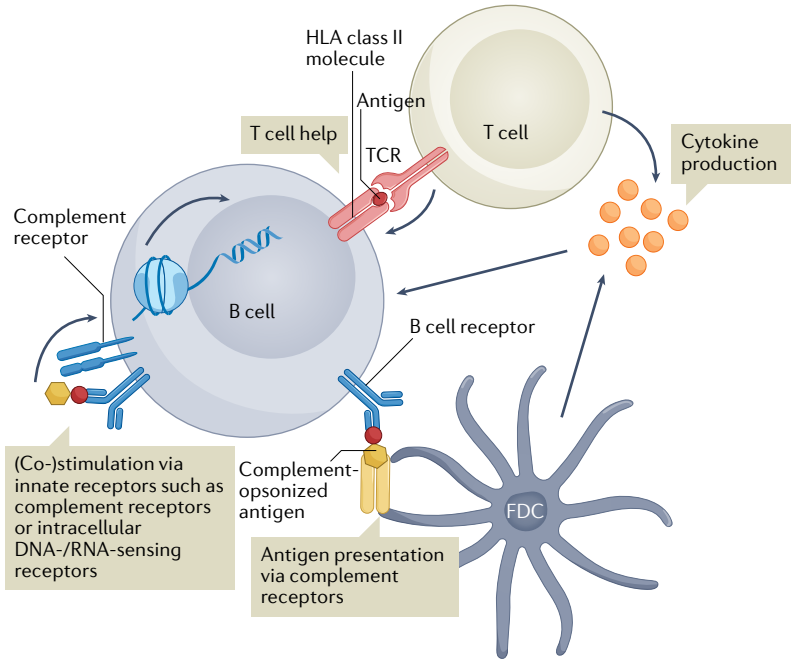


Fig. 2 | **Peripheral checkpoints controlling B cell activation.** As autoreactive B cells escape central tolerance checkpoints in the bone marrow, B cell stimulation needs to be tightly controlled at later stages in the periphery in order to prevent the development of autoreactive B cells. In germinal centre reactions, follicular dendritic cells (FDCs) bind complement-opsonized antigens via complement receptors (such as binding of C3d-opsonized antigen via the complement C3d receptor (CD21)). B cells recognize these antigens but need additional co-stimulation by innate immune stimuli and help from T cells for full activation. In addition, FDC-derived and T cell-derived cytokines (such as IL-6 and IL-21) modify the way in which B cells respond to the antigenic stimulus, thereby inducing different B cell phenotypes and fates. Insufficient presence of such (combinations of) stimuli will not result in productive B cell responses. If the antigen is an RNA-binding or DNA-binding protein, co-stimulatory signals can also be delivered via intracellular RNA-sensing or DNA-sensing receptors.

immune-complexed antigens that have been opsonized (tagged by complement) and hence can be retained through complement receptors CR1 and CR2 (CD35 and CD21)[50]. Because many microbes can activate the complement system, in contrast to most self-proteins, antigen presentation to B cells in itself represents a relevant first checkpoint preventing unwanted B cell responses. In relation to the development of B cells against modified antigens, it is therefore conceivable that the initial triggers are derived from complement activation by microbes that express modified proteins. B cells require additional signals for proper activation when recognizing antigen through the BCR. These co-stimulatory signals act as a second checkpoint and are provided, in conjunction with BCR triggering, by innate immune receptors such as Toll-like receptors and complement receptors CD21 and CD35 (REFS[51,52]). Thus, innate immune recognition of antigens is crucial to the activation of antigen-specific B cells, as both antigen presentation and B cell co-stimulation are promoted by such innate signals. Notably, in this context, many antigenic moieties that are recognized by autoreactive B cells are either linked to molecules that are able to activate innate receptors, or they are directly capable of such activation. These molecules include DNA, RNA and proteins that can interact with these nucleic acids, as reviewed elsewhere[46,53].

A third peripheral checkpoint that autoreactive B cells need to pass to develop into persistent, class-switched cells, is the need to attract help provided by antigen-specific CD4$^+$ T helper (T_H) cells (FIG. 2). Because both central and peripheral tolerance mechanisms restrict the formation of autoreactive T cell responses, the likelihood that both T cells and B cells that are autoreactive to a given antigen are present in an individual is low, so the 'T cell checkpoint' likely represents a formidable barrier. Human T cells that react with citrullinated autoantigens have been found in peripheral blood and joints of patients with RA. Whether these cells function in the development of ACPA-expressing B cells, however, is not known[54–57]. By contrast, T cells that recognize modified proteins of foreign origin could also contribute to the development of B cell autoimmunity. In fact, cross-reactivity of autoantibodies to antigens expressed by microbes or antigens that can provide T cell assistance by enlisting 'non-self-directed' T cells occurs for several autoreactive B cell responses in human autoimmune diseases[58–61]. Examples include the recognition of commensal orthologues of the human autoantigen Ro60 in the pathogenesis of systemic lupus erythematosus, cross-reactivity of autoantibodies towards non-orthologous mimotopes of the autoantigen β2-glycoprotein I in models of antiphospholipid syndrome and cross-reactivity of anti-Epstein–Barr virus nuclear antigen 1 antibodies with the central nervous system protein glial cell adhesion molecule in patients with multiple sclerosis[62]. In coeliac disease, a highly disease-specific autoreactive B cell response to tissue transglutaminase is supported by a T_H cell response directed against a non-self-antigen, modified gluten[63,64], which forms complexes with tissue transglutaminase. These findings demonstrate that autoreactive B cell responses can be generated and can persist in the absence of an autoreactive T cell response if help can be recruited from T cells directed against non-self-antigens (FIG. 3). Such antigens may be either directly recognized through cross-reactivity of T cells and/or B cells or through molecular antigen mimicry, or indirectly recognized through formation of complexes of self-antigens and non-self-antigens (a mechanism originally described as a 'carrier effect')[65].

In RA, cross-reactivity against several PTM antigens is a feature that is already present in the ACPA IgM compartment, which indicates that the first-responding, IgM-expressing B cells can recognize a diverse array of modified antigens[41]. Therefore, it is challenging to define or identify an inciting antigen underlying the initial activation of AMPA-expressing B cells, or the antigen(s) responsible for the recruitment of T cell help. Because AMPAs are cross-reactive to many different modified proteins, these antigens do not have to be the same at all disease stages or at all locations in the body, or between different patients. Similarly, T cells can recognize modified self-proteins as well as non-self-proteins[55–57,66–68], and it is unclear whether one or both types of T cell reactivity are involved in the provision of help to AMPA-expressing B cells. Nevertheless,

the boundaries and checkpoints involved in B cell activation might provide insights into the nature of the antigens involved in initiation and propagation of AMPA responses. In this respect, it is striking that AMPAs can recognize proteins linked to DNA, such as histones, as well as many other modified proteins expressed by microbes. In doing so, AMPA-expressing B cells will not only be triggered by modified antigens, but are also able to receive signals via innate immune receptors such as TLRs, CD21 and CD35. Likewise, as both DNA and microbes can be targets for complement activation[69–71], they can also be captured by complement receptors on FDCs, enabling efficient presentation to B cells. Moreover, microorganism-directed T_H cell activity could be recruited by responding B cells for further maturation of emerging AMPA responses.

In conclusion, several checkpoints controlling B cell tolerance need to be overcome in the development of autoreactive B cell responses. Various distinct characteristics of AMPA-specific autoimmune responses, including cross-reactivity (enabling T cell help) and V-domain glycosylation (described in detail below), and the recognition of DNA-binding or RNA-binding post-translationally modified proteins, such as histones, could enable the AMPA response to pass these checkpoints, thereby providing the immunological basis for subsequent disease development.

RA autoantibody-response risk factors

The mechanisms underlying the development of the autoreactive AMPA response should be viewed in the context of the temporal development of RA and the risk factors associated with this disease (TABLE 1). Generally, the transition over time from health to the onset of seropositive RA is seen as a step-wise process, in which several phases can be discerned[72]. In the first phase, although there are not yet signs of autoimmunity or disease, the presence of genetic and environmental risk factors predisposes some individuals to disease. In the next step, autoantibodies appear as a sign of systemic autoimmunity associated with RA, which is still asymptomatic. Subsequently, symptoms develop and many patients progress through arthralgia (pain without joint swelling) and undifferentiated arthritis (arthritis not yet typical for RA or not yet fulfilling RA classification criteria) to the symmetrical polyarthritis that is characteristic of RA. Although not all patients progress through these phases, this step-wise model provides a useful framework for placing the effects of risk factors and immunological events in a temporal context. Furthermore, these phases are key to designing interventional trials aimed at preventing RA onset[73].

A wealth of genetic and environmental risk factors are associated with RA[74,75], and most of them specifically or predominantly predispose to seropositive disease.

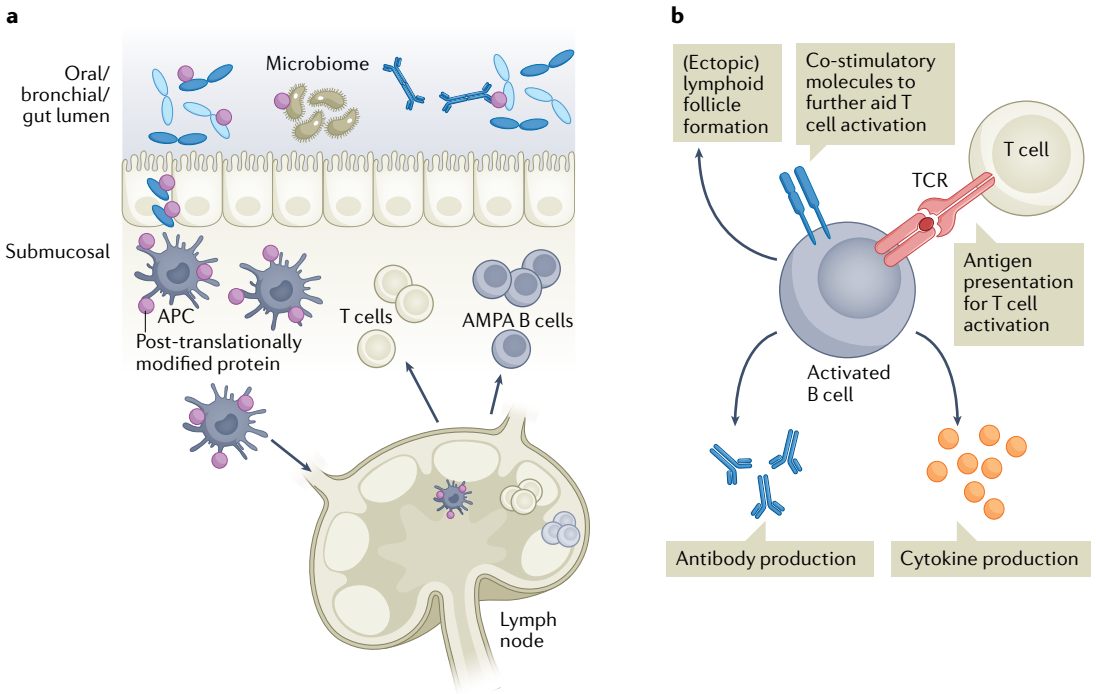


Fig. 3 | **Potential induction of autoreactive B cells at mucosal sites and their possible contribution to rheumatoid arthritis. a** | Post-translational modification of proteins is a common feature of many microbial species. Upon gut-barrier leakage or active uptake, such antigens can reach mucosal antigen-presenting cells (APCs), which may recruit T cells that react with microbial antigens. Modified proteins, such as those with acetylation of lysine residues, are likely to also be recognized by cross-reactive anti-modified-protein antibodies (AMPAs) and AMPA-expressing B cells. Because these B cells can themselves internalize and present microbial antigens, they can recruit microbial antigen-reactive T cell help, leading to their activation, despite their autoreactivity. **b** | Activated B cells exert various effector functions that may be relevant to the initiation and maintenance of synovial inflammation, including secretion of inflammatory and regulatory cytokines, formation of tertiary lymphoid structures (follicles), activation of T cells via antigen presentation and expression of co-stimulatory molecules, and differentiation into antibody-secreting cells.

Table 1 | **Features of two distinct phases of pathogenic autoimmunity in rheumatoid arthritis**

Feature	Onset and development phase of autoimmunity	Maturation phase of autoimmunity
Risk factors	Environmental (such as smoking) or microbiomic factors	Genetic, such as HLA shared epitope alleles; partly environmental (smoking, microbiomic factors)
Events	Enhanced availability of post-translational modifications on proteins; break of tolerance (T cells providing help to B cells); B cells producing 'non-matured' antibodies	HLA shared epitope-restricted T cells provide help to B cells; further maturation with further shaping of a cross-reactive anti-modified-protein antibody repertoire; production of 'matured' autoantibodies (extended cross-reactivity, enhanced V-domain glycosylation)
Clinical correlates	Healthy individual with detectable autoantibodies	Transition from healthy to symptomatic (arthralgia or arthritis)

These findings are corroborated by studies of the heritability of RA[76], which is defined as the proportion of RA variance that is attributable to additive genetic effects. The heritability of seropositive disease is consistently higher than that of seronegative disease, providing a compelling argument for differences in disease mechanisms underlying seropositive and seronegative RA.

Rather than providing a comprehensive overview of all risk factors for RA, the following paragraphs concentrate on the most important factors in the context of the developing autoimmune B cell and autoantibody responses in seropositive disease. According to the summary of the phases of disease development described above, a distinction can be made as to whether risk factors contribute to the initial development of autoantibodies, or to the later onset of disease. First important insights in this respect were gleaned from a Swedish study of twins[77], in which the most important environmental risk factor (smoking) was mainly associated with the development of autoantibodies, whereas the most important genetic risk factor (*HLA-DRB1* shared epitope alleles) affected the later stage in which ACPA-positive individuals ultimately developed arthritis. Similarly, protective *HLA-DRB1*13* alleles mainly affect the onset of RA in ACPA-positive healthy individuals[78], corroborating the notion that T cell factors have a role in the transition from an autoantibody-positive healthy to diseased state, as described in more detail below. Notably, in a study that included the intermediate phase of arthralgia, smoking was again associated with the development of autoantibodies, whereas the onset of symptoms (arthralgia) and progression to inflammatory arthritis were associated with the presence of HLA shared epitope alleles[79].

The mechanisms underlying the varied temporal effects of the most important risk factors in RA are the subject of several hypotheses. The effect of many environmental risk factors is often ascribed to their influence on the presence or quantity of post-translationally modified proteins[80]. Smoking, for example, leads to accumulation of citrullinated proteins in human bronchoalveolar lavage specimens[81], which could represent an increase in the availability of modified (auto)antigens that might facilitate a break in immune tolerance. A similar mechanism is proposed for periodontitis, the prevalence of which is associated with RA, and in which the citrullinating bacterium *Porphyromonas gingivalis* has an important role[27,82]. Likewise, alteration of the intestinal microbiome in patients with RA[83,84] might increase exposure to modified proteins, because enzymatic activity of the microbiome can lead to PTM, both in bacteria and in the host[85]. Exposure to agents such as smoke leads to damage at mucosal sites, which could enable the recruitment of innate immune mechanisms and co-stimulation of the adaptive immune response. These findings all suggest that the autoimmune response underlying RA starts at mucosal sites, and this concept is known as the 'mucosal origins hypothesis'[86] (FIG. 3). Despite the beauty of this hypothesis, some caveats should be noted. For example, the link between smoking and citrullination is challenged by results demonstrating that smoking in RA is associated with the presence of RF rather than ACPA[87,88]. Nevertheless, the mucosal origins hypothesis provides an interesting framework to facilitate understanding of the initiation of RA-associated autoimmunity.

The most important genetic risk factors for RA (the HLA shared epitope alleles) mainly affect the transition from the healthy autoantibody-positive state to the onset of seropositive disease. This phase is also characterized by a marked evolution of the autoimmune and autoantibody responses, suggesting that the HLA risk factors mainly affect maturation of the autoimmune response, for example, by affecting T cell help to AMPA-expressing B cells, enabling these B cells to produce more 'evolved' AMPAs that have undergone isotype switching and SHM. Notably, this evolution might affect cross-reactivity between different protein modifications and between different proteins carrying the same modification, both of which are important features of a mature AMPA response[16,19,89]. In line with this hypothesis, HLA shared epitope alleles are associated with the recognition of particular citrullinated epitopes[90–92], as well as with the distinct ACPA characteristic of enhanced V-domain glycosylation[93]. However, the presence of ACPAs of the IgG isotype in healthy individuals and pre-disease[23] indicates that the ACPA response has undergone isotype switching, and hence received T cell help, in this early phase as well. These observations implicate at least two different T cell responses in full maturation of the ACPA response: an early or initial T cell response that is not associated with HLA shared epitope alleles, and a later HLA shared epitope-restricted T cell response. In the latter part of this scenario, cross-reaction of AMPAs with several modified antigens might facilitate the mobilization of additional T cell help directed against modified antigens presented by HLA shared epitope molecules.

In summary, the step-wise model of the development of RA via different phases provides a powerful framework. A key aspect in this model is the pre-disease maturation of the autoimmune response, which is described in the following section.

Pre-RA autoreactive B cell responses

Isotype usage, epitope recognition and avidity. After the emergence of autoimmunity, as evidenced by the presence of RA-associated autoantibodies in otherwise healthy individuals, the autoimmune response undergoes a clear evolution, similar to that of T cell-dependent B cell responses, which undergo repeated germinal centre reactions. Serology results indicate that this maturation phase includes rising autoantibody levels[22], isotype switching[94] and recognition of an increasing number of epitopes[30,95]. Although multiple studies[30,96,97] have investigated epitope spreading prior to RA onset, a primary culprit for an initiating antigen or PTM has not emerged, possibly because AMPA responses can be cross-reactive with multiple antigens (FIG. 1). Similarly, the modified proteins that cause the break of tolerance might differ between individuals. Notably, avidity maturation of the ACPA response seems to be limited in all phases of disease development, indicating that in this case, avidity maturation and isotype switching are uncoupled, and that the further selection of citrullinated antigen-directed B cells is not primarily driven by the affinity of the BCR to its antigen.

In line with the mucosal origins hypothesis, researchers have investigated the presence and emergence of IgA AMPAs, looking for clues of a possible intestinal origin of the AMPA response. Although IgA AMPAs, similar to IgG and other isotypes, can indeed develop prior to disease onset[23,89], a clear pattern of pre-disease appearance of autoantibody isotypes has not emerged. Notably, evidence indicates that IgA immune complexes induce osteoclast-mediated bone resorption[98]. Although this property might well be a characteristic of IgA antibodies per se, rather than IgA autoantibodies, these findings nonetheless elucidate an interesting feature of this isotype, especially in the context of RA. Investigations of whether the IgA autoantibodies in RA might be associated with baseline disease activity, treatment response or radiographic progression have not revealed specific associations, but instead indicate that the overall breadth of the isotype response can be relevant to the disease course[99,100].

On the basis of the evaluation of autoantibodies and their characteristics, the evolution of the autoimmune response seems to be complete at RA onset. Likewise, further maturation of the secreted autoantibody repertoire is limited in the phases in which the clinical phenotype progresses from arthralgia through undifferentiated arthritis to RA[30,101]. These observations do not preclude the possibility that dynamic, pathogenically relevant changes to the B cell compartment, its phenotype, activation state and localization in different compartments could be ongoing at this stage. However, they suggest that intervention trials with the intention of preventing RA by prohibiting the maturation of the immune response will be most successful in the very early phases, before patients experience any joint complaints. The fact that patients with arthralgia already possess a fairly mature autoimmune response raises doubts as to whether intervention in that phase, subsequent to the establishment of a mature, presumably long-lived memory B cell and plasma-cell compartment, will be able to permanently reverse this process, unless this compartment can specifically be targeted[102].

V-domain glycosylation. The B cell response directed against citrullinated antigens generates antibodies that are heavily glycosylated in the V domain[103] (FIG. 4). Practically all ACPA IgG molecules (>90%) found in the serum of patients with established RA carry carbohydrate structures in this particular region[103,104]. This extensive glycosylation is remarkable, as it clearly differentiates ACPA IgG from non-autoreactive serum IgG. In serum from patients with RA, only 10–25% of circulating, non-ACPA IgG molecules carry similar V-domain glycans, which is comparable with IgG molecules in healthy individuals[104]. Protective IgG in patients with RA (such as antibodies against tetanus toxoid, a vaccine antigen that mainly induces high-affinity IgG antibodies) also does not exceed this level of V-domain glycosylation[105]. Although glycans are typically present in the C_H2 domain of the Fc tail of every IgG molecule, their presence in the V domain in such abundance is highly unusual, and the only similar observation so far occurs in certain B cell malignancies[106]. Consequently, the accumulation of these glycans raises questions as to their role in the development and function of the ACPA B cell response.

Glycosylation in the ACPA IgG V domain involves fully developed, N-linked, biantennary glycans, with one or two terminal sialic acid residues, one core fucose residue and, frequently, one bisecting *N*-acetylglucosamine[104] (FIG. 4). Most ACPA IgGs carry one N-glycan in each of the two identical antibody V domains (one per Fab arm), but ACPA IgGs carrying up to eight V-domain glycans (four per Fab arm) also occur[107–109]. Notably, N-glycans are enzymatically linked to the antibody backbone during protein folding in the endoplasmic reticulum and become structurally modified in the Golgi apparatus[110]. Their covalent linkage requires a permissive amino acid triplet in the protein backbone of the antibody molecule, known as the N-glycosylation consensus sequence (N-X-S/T, with X indicating any amino acid except proline)[110,111]. ACPA IgG expressed by B cells have such sequons in the V domain, distributed over all framework regions and most complementarity-determining regions[109]. Hence, V-domain glycans are generated within the B cell during the structural development of the antibody molecule and are present in BCRs expressed as membrane-bound molecules on the B cell surface. Depending on their location in the antibody molecule, such glycans can affect antigen binding[112–117]. Computational modelling based on ACPA-expressing B cell-derived BCR sequences and data from related crystal structures suggests that most glycans of the ACPA IgG V domain are located on the outside of the antibody molecule[39,108]. Notably, as has been found for other V-domain-glycosylated IgG,

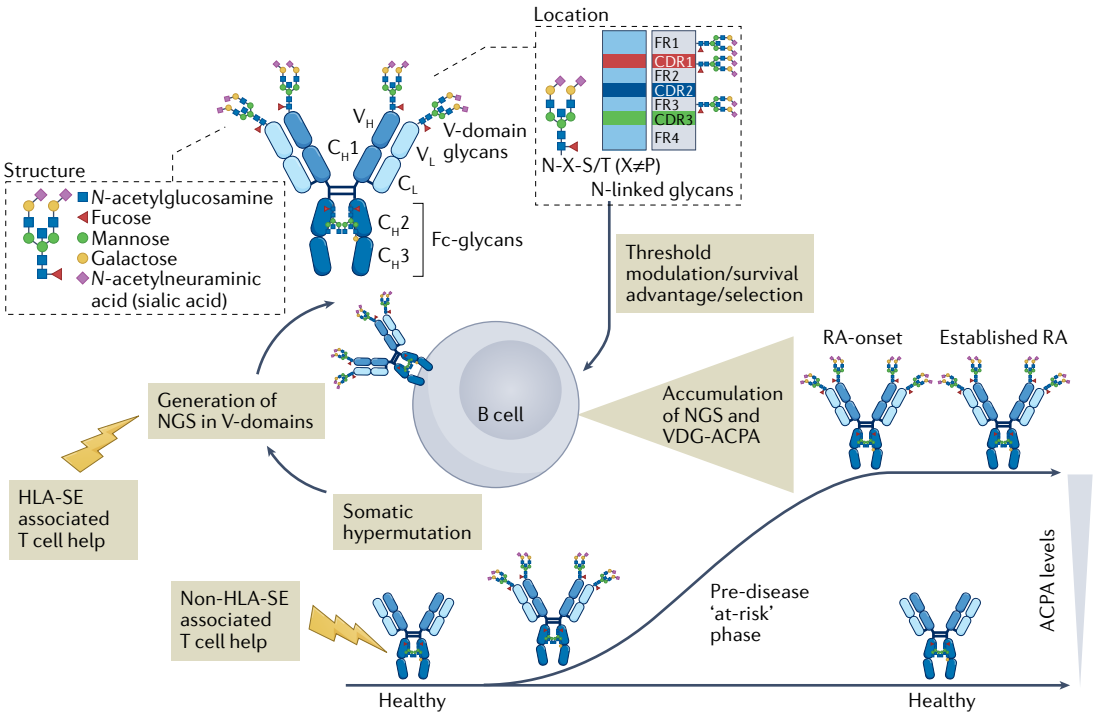


Fig. 4 | **Development and structural features of ACPA V-domain glycosylation.** V-domain glycans (VDGs) are found abundantly on anti-citrullinated protein antibody (ACPA) IgG in established rheumatoid arthritis (RA). VDGs are present on an estimated 93% of ACPA IgG in RA. VDGs vary in structure and composition of subunits. A representative biantennary glycan structure, with two terminal sialic acid residues, one core fucose residue and one bisecting *N*-acetylglucosamine residue, is shown here. VDGs require the presence of N-glycosylation sites (NGSs, which have the consensus sequence N-X-S/T, with X indicating any amino acid except proline) in the antibody V domain, and these NGSs are generated within framework regions (FRs) and complementarity-determining regions (CDRs) of the V domain during the process of somatic hypermutation that is induced by T cell-dependent B cell stimulation events. Longitudinal studies in the pre-RA phase indicate that this T cell–B cell interaction is governed by HLA-shared epitope (SE)-restricted T cells, in contrast to the initial T cell response that is required to generate isotype-switched ACPAs and anti-modified-protein antibodies (AMPAs). In the pre-RA phase, V-domain glycosylated ACPA IgG accumulate towards the onset of RA. The degree of V-domain glycosylation in this phase is associated with the risk of clinical transition from asymptomatic autoimmunity to symptomatic RA.

ACPA IgG N-glycosylation sequons are infrequent in CDR3, which is the region primarily involved in antigen binding[109,118]. However, because N-linked glycans are large, flexible structures, it is possible that V-domain glycans can reach and cover the antigen-binding groove, thereby variably modulating antigen recognition and, possibly, subsequent signalling of the ACPA BCR. This possibility has now been confirmed by extended structural and crystallographic analysis[119]. Moreover, in studies employing immortalized B cell lines engineered to express identical BCRs with and without V-domain glycans, the presence of glycans lowered the threshold for B cell activation by decreasing BCR downmodulation on the cell surface, among other possible mechanisms[119]. ACPA BCR V-domain glycans might influence the structural organization of the BCR on the cell surface, either because of their size (steric effects) or by interaction with carbohydrate-binding molecules on the cell surface (in *cis*) or on neighbouring cells (in *trans*). Whether and how V-domain glycans also modulate effector functions of secreted ACPA IgG molecules is less evident. V-domain glycans might, generally, enhance the stability of IgG molecules[120], and depending on their location and charge, they could also conceivably affect binding of complement components or binding to Fcγ receptors, including the neonatal Fc receptor that is responsible for the serum half-life of human IgG. The relevance of such effects for in vivo antibody function in the context of RA, however, is not yet clear.

Although the functional consequences of V-domain glycans for ACPA effector functions remain to be explored, it is intriguing to postulate that they may be involved in the generation, maturation and survival of ACPA-expressing B cells. In fact, only a few genes for V, D and J segments (the components of the V domain of antibody heavy and light chains) encode N-glycosylation sequons in their germline configurations[105,121]. Analyses of ACPA IgG BCR sequences revealed that none of these germline-encoded gene segments is responsible for the extensive presence of V-domain glycans that is observed on ACPA IgG[109]. Instead, all sequons observed so far were generated by somatic mutations, thereby reflecting an active, dynamic process for the generation of V-domain glycans, which is possibly linked to the maturation of the ACPA B cell response. SHM occurs primarily in germinal centres under the influence of T cell help provided to B cells. Hence, it is likely that the accumulation of N-glycosylation sequons in ACPA IgG V domains reflects the process of repetitive passaging of ACPA-expressing B cells through germinal centres

as part of the maturation of the ACPA B cell response (FIG. 4). Indeed, ACPA IgMs in serum do not show a similar accumulation of V-domain glycans as is observed for ACPA IgGs[122]. Instead, the degree of ACPA IgG V-domain glycosylation is associated with the presence of HLA shared epitope alleles in the pre-disease phase, indicating that shared epitope-restricted T cells may indeed drive this process[93,123]. Nonetheless, germinal centre reactions, SHM and class-switch recombination under the governance of T cells are natural processes that in most humoral immune responses do not lead to this extensive accumulation of V-domain glycans. Also, for ACPA IgG, the accrual of V-domain glycans is not merely the result of the accumulation of somatic mutations. In fact, the numbers of N-glycosylation sequons do not correlate with mutation rates in ACPA IgG BCRs[122]. Because the affinity of ACPA IgG for modified antigens shows little maturation despite extensive SHM, it is conceivable that ACPA-expressing B cells are primarily selected for the presence of V-domain glycans, rather than for high-affinity antigen recognition[124]. The observation that citrullinated protein-directed BCRs harbouring V-domain glycans stay on the B cell surface longer than their non-glycosylated counterparts, and that the presence of these glycans can lead to enhanced B cell activation upon antigen encounter, support this notion[119]. Hence, modulation of the activation threshold might enable ACPA-expressing B cells to survive and to bypass classical mechanisms of selection in the presence of continuous autoantigen exposure. As such, V-domain glycosylation could be a part of a more general mechanism that modulates and balances BCR signals, enabling autoreactivity to evolve. Indeed, enhancement of the numbers of V-domain N-glycans also occurs in other autoreactive B cell responses, albeit not to the same extent as in ACPA IgG glycosylation[105,106,125]. Whether all AMPA IgGs are V-domain glycosylated to this extent is difficult to determine in this context, given the broad cross-reactivity of ACPAs, and might require the identification of large monoclonal and polyclonal AMPA repertoires representing a variety of disease stages and amounts of cross-reactivity. To what extent structural determinants of the antigen are relevant in this process of selection, and whether the repetitive nature of antigenic stimulation and the type of T cells involved are important, also remain to be further elucidated[105,118]. However, the concept delineated above might explain why V-domain glycans accumulate in ACPA IgG-expressing B cells. In addition, the abundance of ACPA IgG V-domain glycans in all patients with RA suggests that the presence of such glycans is a prerequisite for disease development.

Multiple observations indicate that the ACPA B cell response matures prior to the onset of clinical signs and symptoms of arthritis. In line with this notion, the extent of V-domain glycosylation is low in ACPA-positive asymptomatic individuals[93,126]. In this phase, the presence of ACPA IgG is not associated with the presence of HLA shared epitope alleles, and the presence of ACPA in serum is frequently transient[27]. Notably, the amount of ACPA IgG V-domain glycosylation at this stage is associated with the risk of developing RA. In a Canadian cohort of ACPA-positive, asymptomatic individuals, those with abundant glycans in the ACPA IgG V domain were more likely to develop RA than those with low levels of V-domain glycans. V-domain glycosylation does not confer an immediate risk, however, as it can develop some years prior to the onset of symptoms[93,126], and it might instead represent a phenomenon reflecting a maturation state of the ACPA B cell response that facilitates its persistence, followed by HLA shared epitope-associated, T cell-mediated, repetitive stimulation and maturation that can eventually lead to RA.

B cell-response persistence in RA

Multiple studies have investigated the dynamics of the RA autoantibody response following disease onset, and the results generally show an early decrease in the breadth of the autoantibody profile[127,128], followed by long-term stabilization, with slight fluctuations[129]. RF concentrations decrease more prominently than those of ACPAs[130], perhaps reflecting that ACPAs are more often produced by long-lived plasma cells, which are likely to survive in bone-marrow niches or (chronically) inflamed tissues, and which are less susceptible to immunosuppressive treatment. Interestingly, an early decrease in autoantibody levels, or even seroconversion, with levels dropping below the detection limits of conventional assays, is not consistently associated with treatment response or long-term remission[131]. Instead, these changes seem to reflect the intensity of immunosuppression[129], indicating that measurements of autoantibody concentrations post-disease onset do not provide additive information regarding the underlying immunological mechanisms.

The cellular compartment of the AMPA response, its initiators and stimuli, and the factors that maintain its persistence are not well understood. In established RA, ACPA-expressing B cells circulate in the blood at a mean proportion of 1:10,000 B cells. Based on the analysis of monoclonal ACPA derived from these cells, most if not all of these cells express cross-reactive AMPAs[18,32,132,133], and the majority are class-switched, IgG-expressing memory B cells with an activated phenotype characterized by enhanced expression of CD19, HLA-DR and co-stimulatory molecules (FIG. 5). These cells actively proliferate, clonally expand, generate plasmablasts and plasma cells, and phenotypically resemble cells from recall responses evoked by booster vaccination[133–136]. ACPA-expressing B cells are enriched in synovial fluid and are detectable in synovial tissue, with enhanced fractions in the plasmablast and plasma cell compartments[134,137]. In fact, the synovial microenvironment provides conditions that favour their long-term survival[138]. The presence of ACPA-expressing B cells in secondary lymphoid organs such as spleen and bone marrow has not yet been studied, but the limited dynamics of ACPA IgG serum titres that rarely sero-revert, and the high-titre persistence of ACPA IgG after B cell depletion as well as in patients in long-term remission suggests the additional presence of long-lived plasma cells in the bone marrow. Understanding the occurrence and localization of these disease-specific, long-term immunological memory cells will be important, as long-lived

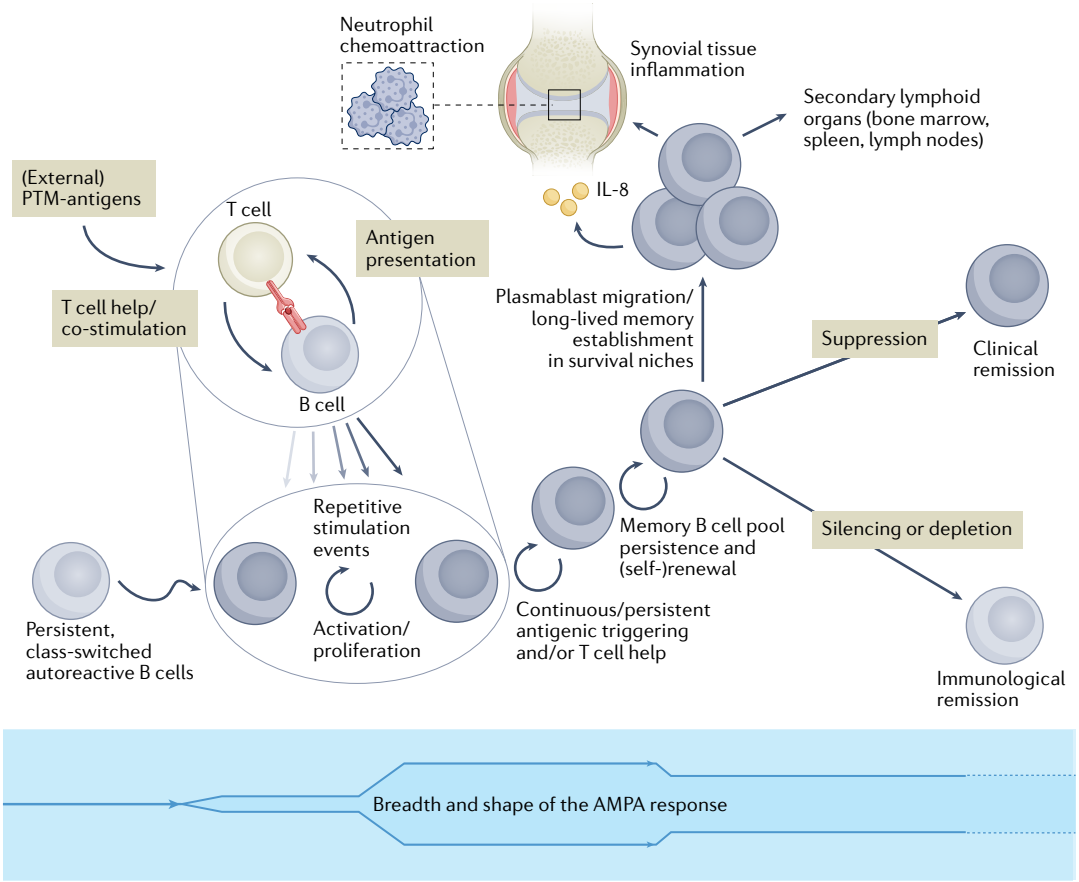


Fig. 5 | **Conceptual framework for autoreactive B cell activation and the maintenance of disease chronicity.** The influence of post-translationally modified (PTM) antigens and T cell help induces the activation of autoreactive, anti-modified-protein antibody (AMPA)-expressing B cells, which circulate primarily as activated, proliferating memory B cells in peripheral blood. In part, these cells differentiate into anti-citrullinated protein antibody (ACPA)/AMPA-secreting plasmablasts and plasma cells, which could migrate towards sites of inflammation. Indeed, ACPA-expressing B cells and plasma cells are enriched in the synovial compartment in rheumatoid arthritis. Notably, these activated, ACPA-expressing B cells express large amounts of IL-8, a strong chemoattractant for neutrophils, the most abundant cell type in synovial fluid. Importantly, most current therapeutic interventions suppress joint inflammation, thereby inducing clinical remission. However, ACPA-expressing B cells frequently remain activated despite clinical remission, which may explain why disease can flare when treatment is stopped. Specific silencing or targeted depletion of ACPA/AMPA-expressing B cells may be required to induce immunological remission, a state of remission that is possibly compulsory to breach disease chronicity and to induce cure.

plasma cells, and in particular persisting memory B cells, may be important factors that determine disease chronicity and that could therefore represent important targets in ACPA-positive RA[102].

ACPA B cells and immunological remission

Despite advances in the control of inflammatory disease activity in RA by early and targeted treatments, ACPA-positive disease is still characterized by low rates of sustained, drug-free remission[139]. As such, RA remains a chronic disease that currently cannot be cured. Patients achieve clinical remission, but disease frequently flares if medication is tapered. Hence, it is conceivable that immunological processes that promote disease remain active in patients despite clinically apparent control of inflammatory disease. To date, the nature of such immunological disease activity is unclear, as are the strategies required to reach immunological remission. In this context, it is intriguing that ACPA-expressing memory B cells in the 'at-risk' arthralgia phase of ACPA-positive autoimmunity proliferate but are otherwise 'quiescent' (non-activated), similar to steady-state memory B cells against recall antigens (such as tetanus toxoid)[134]. By contrast, these cells are strongly activated and proliferate intensely at the onset of arthritis, indicating that although the serologically detectable features of the ACPA response might be fully mature in the 'at risk' arthralgia phase, the cellular component can still undergo dynamic phenotypic and functional changes in the wake of the onset of arthritis. Notably, the highly activated phenotype at arthritis onset is similar to that of B cells induced by recall antigens upon (booster) vaccination. However, B cells in vaccine responses revert to a quiescent, resting (non-proliferating) memory state over time, whereas ACPA-expressing B cells persist as proliferating, activated memory B cells in established RA[132,134,140]. In fact, this phenotype also persists during clinical remission in which synovial inflammation is

therapeutically controlled (FIG. 5). The contrast between quiescence pre-disease, activation at disease onset and persistent, chronic activation in established disease is intriguing as it suggests that the ACPA B cell response mirrors immunological processes that are relevant to the disease course, with the quiescent, non-activated state of ACPA B cells presumably reflecting the state of immunological remission that is required for cure. Whether ACPA-expressing B cells do indeed reflect immunological disease activity is not yet clear. Their contribution to the inflammatory process is likely, however, as these cells express markers that enable communication with T cells, as well as abundant cytokines such as the neutrophil chemoattractant IL-8. Ongoing studies are investigating whether a quiescent phenotype of the ACPA B cell response is therapeutically inducible and maintainable and whether patients that display a quiescent phenotype in clinical remission can safely taper medication (NCT03492658).

Conclusions

Although RA remains a chronic disease, our understanding of the immunological processes that precede its development is constantly increasing. The individual contributions of environmental and genetic factors, as well as detailed features of disease-specific and antigen-specific immune responses, can now be placed in a temporal context with emerging immunological trajectories that need to be traced and delineated for individual patients. In particular, the specific features of the immune response against proteins with PTMs that generate AMPAs, and their striking cross-reactivity, glycosylation and maturation in the context of clinical RA, provide important conceptual advances in relation to initiation and development of the inflammatory process in this disease. Although much still needs to be learned, these developments have enabled the initiation of the first intervention trials in the pre-disease phase. From a pathophysiological perspective, important next steps relate to the identification of potential microbial contributors to disease development, in particular with regard to the HLA shared epitope-restricted T cell response that presumably stimulates and shapes the maturation of AMPA-expressing B cells. Although it is difficult to fully understand how ACPAs, AMPAs and the underlying B cell responses eventually contribute to RA, exploiting the changes in these factors to trace immunological disease activity in RA can enable therapy to be adjusted so that immunological remission comes into reach. Finally, targeting the persistent memory compartment of the AMPA B cell response could be pivotal to reaching the ultimate goal in RA: cure.

Published online 23 May 2022

1. van der Helm-van Mil, A. H., Verpoort, K. N., Breedveld, F. C., Toes, R. E. & Huizinga, T. W. Antibodies to citrullinated proteins and differences in clinical progression of rheumatoid arthritis. *Arthritis Res. Ther.* **7**, R949–R958 (2005).
2. Whiting, P. F. et al. Systematic review: accuracy of anti-citrullinated peptide antibodies for diagnosing rheumatoid arthritis. *Ann. Intern. Med.* **152**, 456–464 (2010). W155-466.
3. Waaler, E. On the occurrence of a factor in human serum activating the specific agglutination of sheep blood corpuscles. 1939. *APMIS* **115**, 422–438 (2007). discussion 439.
4. Edelman, G. M., Kunkel, H. G. & Franklin, E. C. Interaction of the rheumatoid factor with antigen-antibody complexes and aggregated gamma globulin. *J. Exp. Med.* **108**, 105–120 (1958).
5. Fehr, T. et al. Role of repetitive antigen patterns for induction of antibodies against antibodies. *J. Exp. Med.* **185**, 1785–1792 (1997).
6. Newkirk, M. M. Rheumatoid factors: host resistance or autoimmunity? *Clin. Immunol.* **104**, 1–13 (2002).
7. Lu, D. R. et al. T cell-dependent affinity maturation and innate immune pathways differentially drive autoreactive B cell responses in rheumatoid arthritis. *Arthritis Rheumatol.* **70**, 1732–1744 (2018).
8. Falkenburg, W. J. J. et al. Identification of clinically and pathophysiologically relevant rheumatoid factor epitopes by engineered IgG Targets. *Arthritis Rheumatol.* **72**, 2005–2016 (2020).
9. Vaughan, J. H. 1992 Joseph J. Bunim Lecture. Pathogenetic concepts and origins of rheumatoid factor in rheumatoid arthritis. *Arthritis Rheum.* **36**, 1–6 (1993).
10. Willemze, A., Trouw, L. A., Toes, R. E. & Huizinga, T. W. The influence of ACPA status and characteristics on the course of RA. *Nat. Rev. Rheumatol.* **8**, 144–152 (2012).
11. Nienhuis, R. L. & Mandema, E. A new serum factor in patients with rheumatoid arthritis; the antiperinuclear factor. *Ann. Rheum. Dis.* **23**, 302–305 (1964).
12. Schellekens, G. A., de Jong, B. A., van den Hoogen, F. H., van de Putte, L. B. & van Venrooij, W. J. Citrulline is an essential constituent of antigenic determinants recognized by rheumatoid arthritis-specific autoantibodies. *J. Clin. Invest.* **101**, 273–281 (1998).
13. Girbal-Neuhauser, E. et al. The epitopes targeted by the rheumatoid arthritis-associated antifilaggrin autoantibodies are posttranslationally generated on various sites of (pro)filaggrin by deimination of arginine residues. *J. Immunol.* **162**, 585–594 (1999).
14. Aletaha, D. et al. 2010 rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. *Arthritis Rheum.* **62**, 2569–2581 (2010).
15. Dekkers, J. S. et al. Breach of autoreactive B cell tolerance by post-translationally modified proteins. *Ann. Rheum. Dis.* **76**, 1449–1457 (2017).
16. Ioan-Facsinay, A. et al. Anti-cyclic citrullinated peptide antibodies are a collection of anti-citrullinated protein antibodies and contain overlapping and non-overlapping reactivities. *Ann. Rheum. Dis.* **70**, 188–193 (2011).
17. Kampstra, A. S. B. et al. Different classes of anti-modified protein antibodies are induced on exposure to antigens expressing only one type of modification. *Ann. Rheum. Dis.* **78**, 908–916 (2019).
18. Kissel, T. et al. Antibodies and B cells recognising citrullinated proteins display a broad cross-reactivity towards other post-translational modifications. *Ann. Rheum. Dis.* **79**, 472–480 (2020).
19. Sahlstrom, P. et al. Different hierarchies of anti-modified protein autoantibody reactivities in rheumatoid arthritis. *Arthritis Rheumatol.* **72**, 1643–1657 (2020).
20. Steen, J. et al. Recognition of amino acid motifs, rather than specific proteins, by human plasma cell-derived monoclonal antibodies to posttranslationally modified proteins in rheumatoid arthritis. *Arthritis Rheumatol.* **71**, 196–209 (2019).
21. Hensvold, A. H. et al. How well do ACPA discriminate and predict RA in the general population: a study based on 12 590 population-representative Swedish twins. *Ann. Rheum. Dis.* **76**, 119–125 (2017).
22. Nielen, M. M. et al. Specific autoantibodies precede the symptoms of rheumatoid arthritis: a study of serial measurements in blood donors. *Arthritis Rheum.* **50**, 380–386 (2004).
23. Rantapaa-Dahlqvist, S. et al. Antibodies against cyclic citrullinated peptide and IgA rheumatoid factor predict the development of rheumatoid arthritis. *Arthritis Rheum.* **48**, 2741–2749 (2003).
24. Tasliyurt, T. et al. The frequency of antibodies against cyclic citrullinated peptides and rheumatoid factor in healthy population: a field study of rheumatoid arthritis from northern Turkey. *Rheumatol. Int.* **33**, 939–942 (2013).
25. Terao, C. et al. Effects of smoking and shared epitope on the production of anti-citrullinated peptide antibody in a Japanese adult population. *Arthritis Care Res.* **66**, 1818–1827 (2014).
26. van Zanten, A. et al. Presence of anticitrullinated protein antibodies in a large population-based cohort from the Netherlands. *Ann. Rheum. Dis.* **76**, 1184–1190 (2017).
27. Tanner, S. et al. A prospective study of the development of inflammatory arthritis in the family members of indigenous North American people with rheumatoid arthritis. *Arthritis Rheumatol.* **71**, 1494–1503 (2019).
28. Bemis, E. A. et al. Factors associated with progression to inflammatory arthritis in first-degree relatives of individuals with RA following autoantibody positive screening in a non-clinical setting. *Ann. Rheum. Dis.* **80**, 154–161 (2021).
29. van de Stadt, L. A. et al. The extent of the anti-citrullinated protein antibody repertoire is associated with arthritis development in patients with seropositive arthralgia. *Ann. Rheum. Dis.* **70**, 128–133 (2011).
30. van der Woude, D. et al. Epitope spreading of the anti-citrullinated protein antibody response occurs before disease onset and is associated with the disease course of early arthritis. *Ann. Rheum. Dis.* **69**, 1554–1561 (2010).
31. Shi, J. et al. Autoantibodies recognizing carbamylated proteins are present in sera of patients with rheumatoid arthritis and predict joint damage. *Proc. Natl Acad. Sci. USA* **108**, 17372–17377 (2011).
32. Lloyd, K. A. et al. Differential ACPA binding to nuclear antigens reveals a PAD-independent pathway and a distinct subset of acetylation cross-reactive autoantibodies in rheumatoid arthritis. *Front. Immunol.* **9**, 3033 (2018).
33. Yang, X. J. The diverse superfamily of lysine acetyltransferases and their roles in leukemia and other diseases. *Nucleic Acids Res.* **32**, 959–976 (2004).
34. Juarez, M. et al. Identification of novel antiacetylated vimentin antibodies in patients with early inflammatory arthritis. *Ann. Rheum. Dis.* **75**, 1099–1107 (2016).
35. Figueiredo, C. P. et al. Antimodified protein antibody response pattern influences the risk for disease relapse in patients with rheumatoid arthritis tapering disease modifying antirheumatic drugs. *Ann. Rheum. Dis.* **76**, 399–407 (2017).

36. Kongpachith, S. et al. Affinity maturation of the anti-citrullinated protein antibody paratope drives epitope spreading and polyreactivity in rheumatoid arthritis. *Arthritis Rheumatol.* **71**, 507–517 (2019).
37. Nijjar, J. S. et al. The impact of autoantibodies against citrullinated, carbamylated, and acetylated peptides on radiographic progression in patients with new-onset rheumatoid arthritis: an observational cohort study. *Lancet Rheumatol.* **3**, e284–e293 (2021).
38. Ge, C. & Holmdahl, R. The structure, specificity and function of anti-citrullinated protein antibodies. *Nat. Rev. Rheumatol.* **15**, 503–508 (2019).
39. Ge, C. et al. Structural basis of cross-reactivity of anti-citrullinated protein antibodies. *Arthritis Rheumatol.* **71**, 210–221 (2019).
40. Uysal, H. et al. Structure and pathogenicity of antibodies specific for citrullinated collagen type II in experimental arthritis. *J. Exp. Med.* **206**, 449–462 (2009).
41. Reijm, S. et al. Cross-reactivity of IgM anti-modified protein antibodies in rheumatoid arthritis despite limited mutational load. *Arthritis Res. Ther.* **23**, 230 (2021).
42. Lang, J. et al. B cells are exquisitely sensitive to central tolerance and receptor editing induced by ultralow affinity, membrane-bound antigen. *J. Exp. Med.* **184**, 1685–1697 (1996).
43. Nemazee, D. Mechanisms of central tolerance for B cells. *Nat. Rev. Immunol.* **17**, 281–294 (2017).
44. Halverson, R., Torres, R. M. & Pelanda, R. Receptor editing is the main mechanism of B cell tolerance toward membrane antigens. *Nat. Immunol.* **5**, 645–650 (2004).
45. Meffre, E. & O'Connor, K. C. Impaired B-cell tolerance checkpoints promote the development of autoimmune diseases and pathogenic autoantibodies. *Immunol. Rev.* **292**, 90–101 (2019).
46. Reijm, S., Kissel, T. & Toes, R. E. M. Checkpoints controlling the induction of B cell mediated autoimmunity in human autoimmune diseases. *Eur. J. Immunol.* **50**, 1885–1894 (2020).
47. Heesters, B. A., Myers, R. C. & Carroll, M. C. Follicular dendritic cells: dynamic antigen libraries. *Nat. Rev. Immunol.* **14**, 495–504 (2014).
48. Ansel, K. M. et al. A chemokine-driven positive feedback loop organizes lymphoid follicles. *Nature* **406**, 309–314 (2000).
49. Nossal, G. J., Ada, G. L. & Austin, C. M. Antigens in immunity. IV. Cellular localization of 125-I- and 131-I-labelled flagella in lymph nodes. *Aust. J. Exp. Biol. Med. Sci.* **42**, 311–330 (1964).
50. Carroll, M. C. The role of complement and complement receptors in induction and regulation of immunity. *Annu. Rev. Immunol.* **16**, 545–568 (1998).
51. Cumpelik, A. et al. Dynamic regulation of B cell complement signaling is integral to germinal center responses. *Nat. Immunol.* **22**, 757–768 (2021).
52. Leadbetter, E. A. et al. Chromatin-IgG complexes activate B cells by dual engagement of IgM and Toll-like receptors. *Nature* **416**, 603–607 (2002).
53. Elsner, R. A. & Shlomchik, M. J. Germinal center and extrafollicular B Cell responses in vaccination, immunity, and autoimmunity. *Immunity* **53**, 1136–1150 (2020).
54. Chemin, K. et al. A novel HLA-DRB1*10:01-restricted T cell epitope from citrullinated type II collagen relevant to rheumatoid arthritis. *Arthritis Rheumatol.* **68**, 1124–1135 (2016).
55. Feitsma, A. L. et al. Identification of citrullinated vimentin peptides as T cell epitopes in HLA-DR4-positive patients with rheumatoid arthritis. *Arthritis Rheum.* **62**, 117–125 (2010).
56. Pieper, J. et al. Memory T cells specific to citrullinated alpha-enolase are enriched in the rheumatic joint. *J. Autoimmun.* **92**, 47–56 (2018).
57. Snir, O. et al. Identification and functional characterization of T cells reactive to citrullinated vimentin in HLA-DRB1*0401-positive humanized mice and rheumatoid arthritis patients. *Arthritis Rheum.* **63**, 2873–2883 (2011).
58. McClain, M. T. et al. Early events in lupus humoral autoimmunity suggest initiation through molecular mimicry. *Nat. Med.* **11**, 85–89 (2005).
59. Ruff, W. E. et al. Pathogenic autoreactive T and B cells cross-react with mimotopes expressed by a common human gut commensal to trigger autoimmunity. *Cell Host Microbe* **26**, 100–113.e8 (2019).
60. Greiling, T. M. et al. Commensal orthologs of the human autoantigen Ro60 as triggers of autoimmunity in lupus. *Sci. Transl. Med.* https://doi.org/10.1126/scitranslmed.aan2306 (2018).
61. Qian, Y. et al. Cutting edge: Brazilian pemphigus foliaceus anti-desmoglein 1 autoantibodies cross-react with sand fly salivary LJM11 antigen. *J. Immunol.* **189**, 1535–1539 (2012).
62. Lanz, T. V. et al. Clonally expanded B cells in multiple sclerosis bind EBV EBNA1 and GlialCAM. *Nature* **603**, 321–327 (2022).
63. Arentz-Hansen, H. et al. The intestinal T cell response to alpha-gliadin in adult celiac disease is focused on a single deamidated glutamine targeted by tissue transglutaminase. *J. Exp. Med.* **191**, 603–612 (2000).
64. van de Wal, Y. et al. Small intestinal T cells of celiac disease patients recognize a natural pepsin fragment of gliadin. *Proc. Natl Acad. Sci. USA* **95**, 10050–10054, https://doi.org/10.1073/pnas.95.17.10050 (1998).
65. Parker, D. C. The carrier effect and T cell/B cell cooperation in the antibody response. *J. Immunol.* **191**, 2025–2027 (2013).
66. James, E. A. et al. Citrulline-specific Th1 cells are increased in rheumatoid arthritis and their frequency is influenced by disease duration and therapy. *Arthritis Rheumatol.* **66**, 1712–1722 (2014).
67. Song, J. et al. Shared recognition of citrullinated tenascin-C peptides by T and B cells in rheumatoid arthritis. *JCI Insight* https://doi.org/10.1172/jci.insight.145217 (2021).
68. van Heemst, J. et al. Crossreactivity to vinculin and microbes provides a molecular basis for HLA-based protection against rheumatoid arthritis. *Nat. Commun.* **6**, 6681 (2015).
69. Bajic, G., Degn, S. E., Thiel, S. & Andersen, G. R. Complement activation, regulation, and molecular basis for complement-related diseases. *EMBO J.* **34**, 2735–2757 (2015).
70. Holers, V. M. Complement and its receptors: new insights into human disease. *Annu. Rev. Immunol.* **32**, 433–459 (2014).
71. Jiang, H., Cooper, B., Robey, F. A. & Gewurz, H. DNA binds and activates complement via residues 14-26 of the human C1q A chain. *J. Biol. Chem.* **267**, 25597–25601 (1992).
72. Gerlag, D. M. et al. EULAR recommendations for terminology and research in individuals at risk of rheumatoid arthritis: report from the Study Group for Risk Factors for Rheumatoid Arthritis. *Ann. Rheum. Dis.* **71**, 638–641 (2012).
73. Toes, R. E. M. & Raza, K. The autoimmune response as a potential target for tolerance induction before the development of rheumatoid arthritis. *Lancet Rheumatol.* **3**, E214–E223 (2021).
74. Karlson, E. W. & Deane, K. Environmental and gene-environment interactions and risk of rheumatoid arthritis. *Rheum. Dis. Clin. North. Am.* **38**, 405–426 (2012).
75. Okada, Y., Eyre, S., Suzuki, A., Kochi, Y. & Yamamoto, K. Genetics of rheumatoid arthritis: 2018 status. *Ann. Rheum. Dis.* **78**, 446–453 (2019).
76. Frisell, T., Saevarsdottir, S. & Askling, J. Family history of rheumatoid arthritis: an old concept with new developments. *Nat. Rev. Rheumatol.* **12**, 335–343 (2016).
77. Hensvold, A. H. et al. Environmental and genetic factors in the development of anticitrullinated protein antibodies (ACPAs) and ACPA-positive rheumatoid arthritis: an epidemiological investigation in twins. *Ann. Rheum. Dis.* **74**, 375–380 (2015).
78. van Heemst, J. et al. Protective effect of HLA-DRB1*13 alleles during specific phases in the development of ACPA-positive RA. *Ann. Rheum. Dis.* **75**, 1891–1898 (2016).
79. Wouters, F. et al. Determining in which pre-arthritis stage HLA-shared epitope alleles and smoking exert their effect on the development of rheumatoid arthritis. *Ann. Rheum. Dis.* **81**, 48–55 (2022).
80. Klareskog, L. et al. A new model for an etiology of rheumatoid arthritis: smoking may trigger HLA-DR (shared epitope)-restricted immune reactions to autoantigens modified by citrullination. *Arthritis Rheum.* **54**, 38–46 (2006).
81. Makrygiannakis, D. et al. Smoking increases peptidylarginine deiminase 2 enzyme expression in human lungs and increases citrullination in BAL cells. *Ann. Rheum. Dis.* **67**, 1488–1492 (2008).
82. Nesse, W. et al. The periodontium of periodontitis patients contains citrullinated proteins which may play a role in ACPA (anti-citrullinated protein antibody) formation. *J. Clin. Periodontol.* **39**, 599–607 (2012).
83. Scher, J. U. et al. Expansion of intestinal *Prevotella copri* correlates with enhanced susceptibility to arthritis. *eLife* **2**, e01202 (2013).
84. Zhang, X. et al. The oral and gut microbiomes are perturbed in rheumatoid arthritis and partly normalized after treatment. *Nat. Med.* **21**, 895–905 (2015).
85. Zhang, X. et al. Widespread protein lysine acetylation in gut microbiome and its alterations in patients with Crohn's disease. *Nat. Commun.* **11**, 4120 (2020).
86. Holers, V. M. et al. Rheumatoid arthritis and the mucosal origins hypothesis: protection turns to destruction. *Nat. Rev. Rheumatol.* **14**, 542–557 (2018).
87. van Wesemael, T. J. et al. Smoking is associated with the concurrent presence of multiple autoantibodies in rheumatoid arthritis rather than with anti-citrullinated protein antibodies per se: a multicenter cohort study. *Arthritis Res. Ther.* **18**, 285 (2016).
88. Regueiro, C. et al. A predominant involvement of the triple seropositive patients and others with rheumatoid factor in the association of smoking with rheumatoid arthritis. *Sci. Rep.* **10**, 3355 (2020).
89. Ioan-Facsinay, A. et al. Marked differences in fine specificity and isotype usage of the anti-citrullinated protein antibody in health and disease. *Arthritis Rheum.* **58**, 3000–3008 (2008).
90. Mahdi, H. et al. Specific interaction between genotype, smoking and autoimmunity to citrullinated alpha-enolase in the etiology of rheumatoid arthritis. *Nat. Genet.* **41**, 1319–1324 (2009).
91. van der Woude, D. et al. Gene-environment interaction influences the reactivity of autoantibodies to citrullinated antigens in rheumatoid arthritis. *Nat. Genet.* **42**, 814–816 (2010). author reply 816.
92. Verpoort, K. N. et al. Fine specificity of the anti-citrullinated protein antibody response is influenced by the shared epitope alleles. *Arthritis Rheum.* **56**, 3949–3952 (2007).
93. Kissel, T. et al. On the presence of HLA-SE alleles and ACPA-IgG variable domain glycosylation in the phase preceding the development of rheumatoid arthritis. *Ann. Rheum. Dis.* **78**, 1616–1620 (2019).
94. Kokkonen, H. et al. Antibodies of IgG, IgA and IgM isotypes against cyclic citrullinated peptide precede the development of rheumatoid arthritis. *Arthritis Res. Ther.* **13**, R13 (2011).
95. van de Stadt, L. A. et al. Development of the anti-citrullinated protein antibody repertoire prior to the onset of rheumatoid arthritis. *Arthritis Rheum.* **63**, 3226–3233 (2011).
96. Gan, R. W. et al. Anti-carbamylated protein antibodies are present prior to rheumatoid arthritis and are associated with its future diagnosis. *J. Rheumatol.* **42**, 572–579 (2015).
97. Sokolove, J. et al. Autoantibody epitope spreading in the pre-clinical phase predicts progression to rheumatoid arthritis. *PLoS One* **7**, e35296 (2012).
98. Breedveld, A. C. et al. IgA immune complexes induce osteoclast-mediated bone resorption. *Front. Immunol.* **12**, 651049 (2021).
99. de Moel, E. C. et al. Baseline autoantibody profile in rheumatoid arthritis is associated with early treatment response but not long-term outcomes. *Arthritis Res. Ther.* **20**, 33 (2018).
100. van der Woude, D. et al. The ACPA isotype profile reflects long-term radiographic progression in rheumatoid arthritis. *Ann. Rheum. Dis.* **69**, 1110–1116 (2010).
101. Wouters, F. et al. Do autoantibody-responses mature between presentation with arthralgia suspicious for progression to rheumatoid arthritis and development of clinically apparent inflammatory arthritis? A longitudinal serological study. *Ann. Rheum. Dis.* https://doi.org/10.1136/annrheumdis-2020-218221 (2020).
102. Maschmeyer, P. et al. Immunological memory in rheumatic inflammation — a roadblock to tolerance induction. *Nat. Rev. Rheumatol.* **17**, 291–305 (2021).
103. Rombouts, Y. et al. Extensive glycosylation of ACPA-IgG variable domains modulates binding to citrullinated antigens in rheumatoid arthritis. *Ann. Rheum. Dis.* **75**, 578–585 (2016).
104. Hafkenscheid, L. et al. Structural analysis of variable domain glycosylation of anti-citrullinated protein antibodies in rheumatoid arthritis reveals the presence of highly sialylated glycans. *Mol. Cell Proteom.* **16**, 278–287 (2017).
105. van de Bovenkamp, F. S. et al. Adaptive antibody diversification through N-linked glycosylation of the immunoglobulin variable region. *Proc. Natl Acad. Sci. USA* **115**, 1901–1906 (2018).
106. Vletter, E. M., Koning, M. T., Scherer, H. U., Veelken, H. & Toes, R. E. M. A comparison of immunoglobulin variable region N-linked glycosylation in healthy

donors, autoimmune disease and lymphoma. *Front. Immunol.* **11**, 241 (2020).
107. Lloyd, K. A. et al. Variable domain N-linked glycosylation and negative surface charge are key features of monoclonal ACPA: implications for B-cell selection. *Eur. J. Immunol.* **48**, 1030–1045 (2018).
108. Vergroesen, R. D. et al. B-cell receptor sequencing of anti-citrullinated protein antibody (ACPA) IgG-expressing B cells indicates a selective advantage for the introduction of N-glycosylation sites during somatic hypermutation. *Ann. Rheum. Dis.* **77**, 956–958 (2018).
109. Vergroesen, R. D. et al. N-glycosylation site analysis of citrullinated antigen-specific B-cell receptors indicates alternative selection pathways during autoreactive B-cell development. *Front. Immunol.* **10**, 2092 (2019).
110. Kaplan, H. A., Welply, J. K. & Lennarz, W. J. Oligosaccharyl transferase: the central enzyme in the pathway of glycoprotein assembly. *Biochim. Biophys. Acta* **906**, 161–173 (1987).
111. Shakin-Eshleman, S. H., Spitalnik, S. L. & Kasturi, L. The amino acid at the X position of an Asn-X-Ser sequon is an important determinant of N-linked core-glycosylation efficiency. *J. Biol. Chem.* **271**, 6363–6366 (1996).
112. Chuang, G. Y. et al. Removal of variable domain N-linked glycosylation as a means to improve the homogeneity of HIV-1 broadly neutralizing antibodies. *MAbs* **12**, 1836719 (2020).
113. Wallick, S. C., Kabat, E. A. & Morrison, S. L. Glycosylation of a VH residue of a monoclonal antibody against alpha (1–6) dextran increases its affinity for antigen. *J. Exp. Med.* **168**, 1099–1109 (1988).
114. Co, M. S. et al. Genetically engineered deglycosylation of the variable domain increases the affinity of an anti-CD33 monoclonal antibody. *Mol. Immunol.* **30**, 1361–1367 (1993).
115. Khurana, S., Raghunathan, V. & Salunke, D. M. The variable domain glycosylation in a monoclonal antibody specific to GnRH modulates antigen binding. *Biochem. Biophys. Res. Commun.* **234**, 465–469 (1997).
116. Leibiger, H., Wustner, D., Stigler, R. D. & Marx, U. Variable domain-linked oligosaccharides of a human monoclonal IgG: structure and influence on antigen binding. *Biochem. J.* **338**, 529–538 (1999).
117. Tachibana, H., Kim, J. Y. & Shirahata, S. Building high affinity human antibodies by altering the glycosylation on the light chain variable region in N-acetylglucosamine-supplemented hybridoma cultures. *Cytotechnology* **23**, 151–159 (1997).
118. Koers, J. et al. Biased N-glycosylation site distribution and acquisition across the antibody V region during B cell maturation. *J. Immunol.* **202**, 2220–2228 (2019).
119. Kissel, T. et al. Surface Ig variable domain glycosylation affects autoantigen binding and acts as threshold for human autoreactive B cell activation. *Sci. Adv.* **8**, eabm1759 (2022).
120. van de Bovenkamp, F. S. et al. Variable domain N-linked glycans acquired during antigen-specific immune responses can contribute to immunoglobulin G antibody stability. *Front. Immunol.* **9**, 740 (2018).
121. Lefranc, M. P. IMGT, the International ImMunoGeneTics Information System. *Cold Spring Harb. Protoc.* **2011**, 595–603 (2011).
122. Kempers, A. C. et al. The extensive glycosylation of the ACPA variable domain observed for ACPA-IgG is absent from ACPA-IgM. *Ann. Rheum. Dis.* **77**, 1087–1088 (2018).
123. Kissel, T. et al. Genetic predisposition (HLA-SE) is associated with ACPA-IgG variable domain glycosylation in the predisease phase of RA. *Ann. Rheum. Dis.* **81**, 141–143 (2022).
124. Suwannalai, P. et al. Avidity maturation of anti-citrullinated protein antibodies in rheumatoid arthritis. *Arthritis Rheum.* **64**, 1323–1328 (2012).
125. Lardinois, O. M. et al. Immunoglobulins G from patients with ANCA-associated vasculitis are atypically glycosylated in both the Fc and Fab regions and the relation to disease activity. *PLoS One* **14**, e0213215 (2019).
126. Hafkenscheid, L. et al. N-linked glycans in the variable domain of IgG anti-citrullinated protein antibodies predict the development of rheumatoid arthritis. *Arthritis Rheumatol.* **71**, 1626–1633 (2019).
127. Bohler, C., Radner, H., Smolen, J. S. & Aletaha, D. Serological changes in the course of traditional and biological disease modifying therapy of rheumatoid arthritis. *Ann. Rheum. Dis.* **72**, 241–244 (2013).
128. Kastbom, A., Strandberg, G., Lindroos, A. & Skogh, T. Anti-CCP antibody test predicts the disease course during 3 years in early rheumatoid arthritis (the Swedish TIRA project). *Ann. Rheum. Dis.* **63**, 1085–1089 (2004).
129. de Moel, E. C. et al. In rheumatoid arthritis, changes in autoantibody levels reflect intensity of immunosuppression, not subsequent treatment response. *Arthritis Res. Ther.* **21**, 28 (2019).
130. Bos, W. H. et al. Differential response of the rheumatoid factor and anticitrullinated protein antibodies during adalimumab treatment in patients with rheumatoid arthritis. *J. Rheumatol.* **35**, 1972–1977 (2008).
131. de Moel, E. C. et al. In RA, becoming seronegative over the first year of treatment does not translate to better chances of drug-free remission. *Ann. Rheum. Dis.* **77**, 1836–1838 (2018).
132. Pelzek, A. J. et al. Persistence of disease-associated anti-citrullinated protein antibody-expressing memory B cells in rheumatoid arthritis in clinical remission. *Arthritis Rheumatol.* **69**, 1176–1186 (2017).
133. Titcombe, P. J. et al. Pathogenic citrulline-multispecific B cell receptor clades in rheumatoid arthritis. *Arthritis Rheumatol.* **70**, 1933–1945 (2018).
134. Kristyanto, H. et al. Persistently activated, proliferative memory autoreactive B cells promote inflammation in rheumatoid arthritis. *Sci. Transl. Med.* https://doi.org/10.1126/scitranslmed.aaz5327 (2020).
135. Kerkman, P. F. et al. Circulating plasmablasts/plasmacells as a source of anticitrullinated protein antibodies in patients with rheumatoid arthritis. *Ann. Rheum. Dis.* **72**, 1259–1263 (2013).
136. Kerkman, P. F. et al. Identification and characterisation of citrullinated antigen-specific B cells in peripheral blood of patients with rheumatoid arthritis. *Ann. Rheum. Dis.* **75**, 1170–1176 (2016).
137. Corsiero, E. et al. Single cell cloning and recombinant monoclonal antibodies generation from RA synovial B cells reveal frequent targeting of citrullinated histones of NETs. *Ann. Rheum. Dis.* **75**, 1866–1875 (2016).
138. Kerkman, P. F. et al. Synovial fluid mononuclear cells provide an environment for long-term survival of antibody-secreting cells and promote the spontaneous production of anti-citrullinated protein antibodies. *Ann. Rheum. Dis.* **75**, 2201–2207 (2016).
139. Verstappen, M., Niemantsverdriet, E., Matthijssen, X. M. E., le Cessie, S. & van der Helm-van Mil, A. H. M. Early DAS response after DMARD-start increases probability of achieving sustained DMARD-free remission in rheumatoid arthritis. *Arthritis Res. Ther.* **22**, 276 (2020).
140. Frolich, D. et al. Secondary immunization generates clonally related antigen-specific plasma cells and memory B cells. *J. Immunol.* **185**, 3103–3110 (2010).

Author contributions
All authors contributed equally to all aspects of the article.

Competing interests
H.U.S. and R.E.M.T. are mentioned inventors on a patent application relating to ACPA-IgG V-domain glycosylation. D.v.d.W. declares no competing interests.

Peer review information
Nature Reviews Rheumatology thanks A. Radbruch and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.

Publisher's note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Synovial tissue macrophages in joint homeostasis, rheumatoid arthritis and disease remission

Mariola Kurowska-Stolarska[1,2] ✉ *and Stefano Alivernini*[1,3,4,5] ✉

Abstract | Synovial tissue macrophages (STMs) were principally recognized as having a pro-inflammatory role in rheumatoid arthritis (RA), serving as the main producers of pathogenic tumour necrosis factor (TNF). Recent advances in single-cell omics have facilitated the discovery of distinct STM populations, providing an atlas of discrete phenotypic clusters in the context of healthy and inflamed joints. Interrogation of the functions of distinct STM populations, via ex vivo and experimental mouse models, has re-defined our understanding of STM biology, opening up new opportunities to better understand the pathology of the arthritic joint. These works have identified STM subpopulations that form a protective lining barrier within the synovial membrane and actively participate in the remission of RA. We discuss how distinct functions of STM clusters shape the synovial tissue environment in health, during inflammation and in disease remission, as well as how an increased understanding of STM heterogeneity might aid the prediction of clinical outcomes and inform novel treatments for RA.

[1]*Research into Inflammatory Arthritis Centre Versus Arthritis (RACE), Glasgow, UK.*

[2]*Institute of Infection, Immunity and Inflammation, University of Glasgow, Glasgow, UK.*

[3]*Division of Rheumatology, Fondazione Policlinico Universitario A. Gemelli IRCCS, Rome, Italy.*

[4]*Division of Rheumatology, Università Cattolica del Sacro Cuore, Rome, Italy.*

[5]*Immunology Research Core Facility, Gemelli Science and Technology Park (GSTeP), Rome, Italy.*

✉*e-mail: mariola.kurowska-stolarska@glasgow.ac.uk; stefano.alivernini@policlinicogemelli.it*

https://doi.org/10.1038/s41584-022-00790-8

Tissue macrophages are ancient innate immune cells of embryonic or adult bone-marrow origin. Macrophages have various tissue-specific roles that facilitate tissue homeostasis, including engulfment and destruction of pathogens, such as those present in the lung airways during gas exchange. Macrophages also have important roles in the inflammatory response, serving to initiate and then contain sterile injury through the promotion and subsequent resolution of inflammation to re-instate tissue immune-homeostasis[1]. In response to instructive signals, such as tissue damage, resident macrophages are assisted by tissue-infiltrating monocyte-derived macrophages that mediate inflammation. The interplay between tissue-resident and tissue-infiltrating macrophages determines the chronicity of tissue inflammation and the quality of tissue repair[2].

Rheumatoid arthritis (RA) is a common, chronic, inflammatory autoimmune disease that is characterized by articular inflammation, often with subsequent progressive joint damage and disability[3]. Several seminal clinical observations provided the first evidence for a key contribution of macrophages to RA pathogenesis. These included the positive association between the abundance of synovial tissue macrophages (STMs) and the extent of joint damage[4], and the positive association between a high disease activity score in local joints and the abundance of macrophages in the synovial sublining layer[5]. The expression of TNF and IL-6 is also associated with the severity of joint pain[5], and until recently, STMs were principally recognized as the main producers of these pro-inflammatory molecules[6]. However, recent advances in single-cell multi-omics have uncovered unexpected heterogeneity in STM function, revealing a rich atlas of discrete phenotypic clusters among tissue-resident and tissue-infiltrating STMs[7,8]. Together with ex vivo and in vivo studies of STM populations, these data have refined our knowledge of STM biology in healthy and arthritic joints, revealing that while certain STM clusters contribute more towards RA pathogenesis, others phenotypes have a key role in RA remission[8,9].

In this Review, we discuss how distinct functions of different STM clusters shape the synovial tissue environment in health, and how they control RA disease progression and remission. We also discuss the potential origin of different STM clusters and the role of the synovial tissue niche in their local differentiation and spatial distribution. Finally, we discuss how understanding STM heterogeneity might aid in the management of RA and inform novel treatments for RA and other rheumatic diseases.

A functional taxonomy of STMs

Over the past 5 years, human and mouse studies from several groups have advanced our understanding of STMs and their role in joint homeostasis and the inflammatory response[7–10]. Here, we attempt to integrate these data into a single taxonomy (FIG. 1). All human

Key points

- New technologies have identified macrophage populations in both the human and mouse joint synovium, with distinct homeostatic, protective and inflammatory functions.
- Tissue-resident synovial tissue macrophages (STMs) form an immune-protective lining barrier, control the development of experimental arthritis, and actively participate in maintaining RA in remission.
- The progression of RA is associated with phenotypic changes in resident STMs and the influx of monocytes that differentiate into STMs with pro-inflammatory functions, driving chronic pathology.
- Capitalizing on the joint-protective and inflammation-resolving biology of newly identified STM clusters might assist the development of novel therapeutics that are aimed at treating arthritis and maintaining disease remission.
- The relative proportions of STM clusters can predict a flare of arthritis following treatment tapering or cessation and might be a useful component of flare prediction algorithms.

and mouse STMs express the pan macrophage markers CD64, CD11b and CD68 (human) or F4/80 (mouse)[8–10]. Within the human synovium, two main populations of macrophages can be distinguished based on cell membrane expression of MerTK[11], a tyrosine protein kinase receptor involved in efferocytosis and resolution of inflammation (BOX 1), and CD206, a C-type lectin mannose receptor that acts as a pattern recognition receptor for pathogens[12]. Specifically, these populations are MerTK^{pos}CD206^{pos} tissue-resident macrophages and MerTK^{neg}CD206^{neg} tissue-infiltrating macrophages[8]. During arthritis MerTK^{pos}CD206^{pos} STMs acquire expression of CD163. Single-cell transcriptomics (scRNAseq) and multiparameter flow cytometry experiments have revealed that these two STM populations contain multiple transcriptionally distinct phenotypic clusters that differ in function and distribution between different stages of arthritis progression.

Tissue-resident (MerTK^{pos}CD206^{pos}) STMs are the predominant macrophage subtype in healthy human and mouse synovium. Two distinct subpopulations of tissue-resident STMs have been identified (FIG. 1). The first is a TREM2^{pos}CX3CR1^{pos}FOLR2^{pos} cluster[8,9] (referred to as TREM2^{pos} from here on) that forms a protective lining layer in the synovial membrane[9] and is likely to be the equivalent of the CD64^{pos}MHCII^{neg} cluster described in healthy mouse synovium[10]. The second subpopulation is a LYVE1^{pos}FOLR2^{high} cluster (RELMα^{pos} in mouse[9]; referred to as LYVE1^{pos} or RELMα^{pos} from here on) that mostly resides in the synovial sublining layer[8,9] and is ostensibly the equivalent of the mouse CD64^{pos}MHCII^{high} cluster[10]. In turn, hierarchical clustering analysis has revealed that both of these resident STM subpopulations (TREM2^{pos} and LYVE1^{pos}) contain distinct phenotypic clusters, which might reflect diverse activation states[8]. For example, a TREM2^{low} cluster is a discrete activation state of TREM2^{pos} STMs, whereas FOLR2^{pos}ICAM1^{pos} and FOLR2^{pos}ID2^{pos} clusters are an activation state and a lineage precursor of LYVE1^{pos} STMs[8], respectively. A third large subpopulation of myeloid cells in the healthy synovium are cells with dendritic cell phenotype, which are CLEC10A^{pos} in humans, and AQP1^{pos} in the mouse[8,9]. Thus, heterogeneity exists within the tissue-resident STMs.

During joint inflammation, such as in patients with RA and in experimental models of arthritis, a shift in the composition of STM clusters is observed. A key feature of this shift is that the synovial protective lining layer becomes distorted and the tissue-infiltrating population (MerTK^{neg}CD206^{neg}) of STMs becomes predominant, increasing in abundance with disease progression. Like resident macrophages, this tissue-infiltrating STM population contains distinct phenotypic clusters, including a CD48^{pos}S100A12^{pos} cluster[8] (previously described as an M1 population[7]), a CD48^{pos}SPP1^{pos} cluster (equivalent to mouse CCR2^{pos}IL-1β^{pos} STMs[9,10]) and a CD48^{pos}ISG15^{pos} cluster with an interferon signature[8] (previously described as an M4 population[7] and equivalent to the mouse CCR2^{pos}ARG1^{pos} STMs[9,10]). By contrast, during sustained remission in patients with RA, tissue-infiltrating (MerTK^{neg}CD206^{neg}) STM clusters are reduced in number, with restoration of the normal, healthy composition of tissue-resident (MerTK^{pos}CD206^{pos}) TREM2^{pos} and LYVE1^{pos} STM subpopulations[8].

The abundance of distinct STM populations seems to be dependent on the type of joint disease. Unlike in RA, non-inflammatory, mechanical stress-induced osteoarthritis (OA) is characterized by a high abundance of tissue-resident (MerTK^{pos}CD206^{pos}) STMs (previously identified as FOLR2^{pos}[13] and an M2 population[7]), compared with the healthy synovium. However, whether the transcriptomic signature of this STM population differs from that in healthy tissue, and whether it contributes to hallmark OA pathological conditions such as cartilage degradation, remains unknown. STM clusters are also poorly characterized in other chronic inflammatory joint diseases, such as psoriatic arthritis (PsA). Mass cytometry analysis of synovial fluid from patients with PsA has identified the robust presence of a myeloid cluster, characterized by high expression of the bone-remodelling mediator osteopontin (SPP1)[14], that resembles the tissue-infiltrating CD48^{pos}SPP1^{pos} cluster observed in active RA[8]. Further analyses, including single-cell transcriptomic and proteomic studies, are needed to elucidate the shared pathogenic roles of different STM phenotypes in RA, OA and PsA, as well as those that contribute to pathological conditions that are unique to these diseases, such as new bone formation and tendinopathy in patients with PsA.

Distinct STM clusters in health and disease

Healthy homeostasis. The synovial membrane is a highly specialized, multifunctional structure consisting of an inner lining and a sublining layer (FIG. 2). The inner lining is thin (<2 mm in radiocarpal joints[15]) but highly cellular, composed of tissue-resident TREM2^{pos} STMs[8,9,16]. This STM population interacts closely with lining-layer synovial fibroblasts (PRG4^{pos}PDPN^{pos}CD90^{neg})[17] that produce components of synovial fluid, such as lubricin and hyaluronic acid, to lubricate the joint during movement. In line with a protective role of the lining layer of the synovial membrane, mouse and human TREM2^{pos} STMs express tight-junction proteins that enable the formation of a physical epithelial-like barrier, protecting against pathogens and controlling the apparent influx

Disease activity score
A composite index to quantify RA activity, calculated with a formula including painful and swollen joints (over 44 or 28 joints), inflammatory lab tests (erythrocyte sedimentation rate or C-reactive protein) and patients' Global Health evaluation.

Synovial membrane
Specialized connective tissue that lines the inner surface of capsules of synovial joints, tendon sheath and bursae.

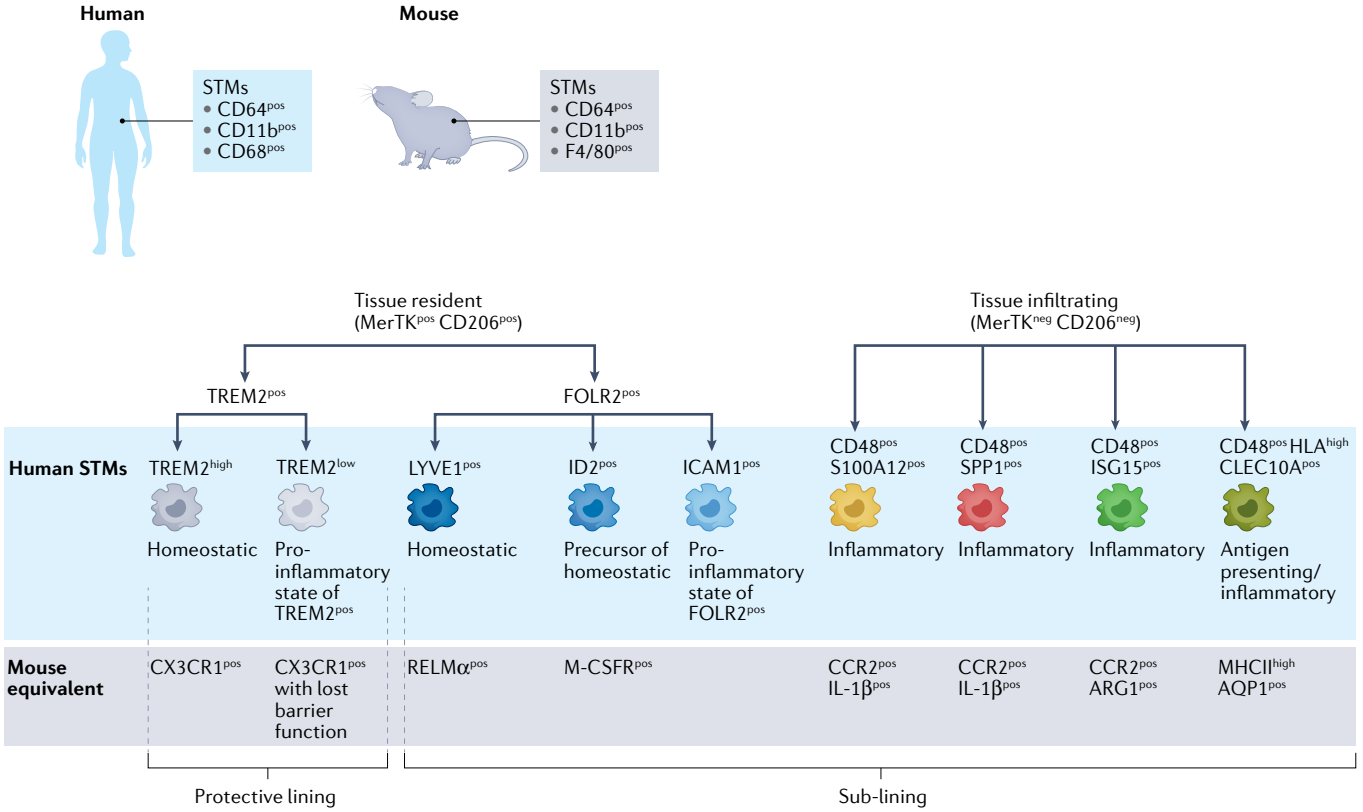


Fig. 1 | **A novel taxonomy of human and mouse STMs defined by single cell omics.** Schematic showing human and mouse synovial tissue macrophage (STM) heterogeneity, revealed by single-cell transcriptomics in Alivernini et al.[8] and Culemann et al.[9]. Human and mouse STMs are clustered based on their transcriptomics, functions and spatial localization within the synovial membrane, in health, rheumatoid arthritis and experimental models of arthritis.

of inflammatory cells[8,9]. In addition, of all known STM clusters, they constitutively express the highest levels of defensins, such as defensin B1, as well as complement proteins such as C1Q[8], which likely reflects their local role in preventing infections and in clearing damaged tissue to prevent rapid cartilage degradation, such as that described in patients with septic arthritis[18]. Consistent with such a function is the finding that both human and mouse TREM2^{pos} STMs express a wide range of phagocytic receptors, including AXL, MARCO and TIMD4 (REFS[8,9]), and constitutively express enzymes involved in phagocytosis (such as lysosomal cathepsins)[8]. Together, these findings suggest that the inner lining STMs have an important role in engulfing apoptotic cells during homeostatic tissue remodelling and removal of dying short-lived inflammatory cells to prevent sterile inflammation[8,9,19].

In addition to their phagocytic function, the regulatory transcriptome of TREM2^{pos} STMs suggests a role in actively restraining inflammation. Pathways enriched in this macrophage population are related to MerTK and AXL, tyrosine kinase receptors that inhibit cytokine- and Toll-like receptor (TLR)-induced pro-inflammatory responses. Additional upregulated pathways include retinoic acid and VSIG4 (a PDL1-like molecule) pathways that inhibit effector T cell activation while facilitating the expansion of regulatory T cells[8,9]. Moreover, of all known STM clusters, TREM2^{pos} STMs have the highest expression levels of receptors and enzymes involved in attenuation of inflammation, such as those related to lipid binding and metabolism (e.g. APOE-binding receptor TREM2 (REF.[8])) and the production of lipid mediators (e.g. resolvin D1)[8]. In this respect, the TREM2^{pos} STM population might share the same tissue function as that of TREM2^{pos} adipose tissue macrophages, the disruption of which leads to adipose tissue inflammation and the development of insulin resistance[20]. Accordingly, data from experimental mouse models suggest that a reduced abundance of lining-layer STMs precedes the development of spontaneous arthritis[21]. In summary, TREM2^{pos} STMs form a protective barrier that maintains synovial lining-layer integrity and limits the development of sterile and pathogen-induced inflammation.

The sublining layer (interstitium) of the synovium contains loose, adipocyte-rich connective tissue and sublining fibroblasts (CD90^{pos}), which provides support to the lining layer[7,17,22]. Sympathetic and sensory nerves, as well as blood and lymphatic vasculature, are also present in the sublining layer, providing a link between the synovium and systemic responses to changes within the tissue environment[16]. Across several tissues, the healthy interstitium contains at least two distinct macrophage clusters: nerve-associated macrophages (NAMs) and perivascular macrophages. NAMs, localized adjacent to the sympathetic nerves, are tissue-resident macrophages characterized by low LYVE1 and high MHCII expression. Studies in mice have identified an important functional interaction

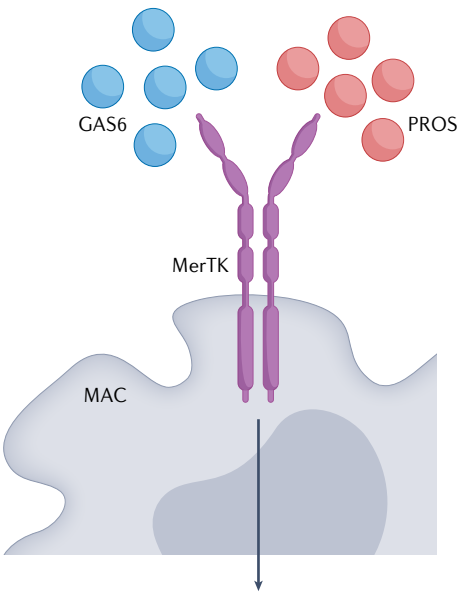
between nerves and NAMs that prevents excessive inflammation in tissues, such as lung, dermis and heart[23,24]. Indeed, NAMs have an immunoregulatory phenotype, in that they express high levels of IL-10 (REFS[23,24]), are potent inducers of regulatory T cells[23] and their depletion in mouse lung leads to an excessive inflammatory response during viral infections[24]. In addition, NAMs can provide essential cues for nerve homeostasis, suggesting two-way communication between macrophages and the nerve. NAMs in the gut produce bone morphogenic protein 2 (BMP2) that binds to its receptor (BMP2R) on enteric neurons and promotes gut neuron-mediated gastrointestinal motility[25]; depletion of NAMs in the gut disrupts normal gut peristatic activity[25]. To date, NAMs have not been formally identified in the human or mouse synovium, although their transcriptomic profile suggests that they might be embedded within the recently described tissue-resident LYVE1^{low}ID2^{pos} STM cluster[8]. Refined characterization of the synovium NAM population is critical to improve our understanding of NAM function and might inform therapeutic strategies targeting the aetiology of joint pain that characterizes inflammatory and non-inflammatory conditions.

Alongside the NAM population, the healthy synovial sublining layer contains a robust LYVE1^{pos} perivascular macrophage cluster (termed RELM1a^{pos} in the mouse) with a transcriptional profile that indicates various regulatory functions. Among all identified STM clusters, the LYVE1^{pos} cluster expresses the highest levels of enzymes involved in haem metabolism (such as HMOX1 and BLVRB)[8], which suggests it has a role in the phagocytosis of erythrocytes, iron recirculation and the prevention of haem-triggered inflammation after blood vessel disruption or injury[26]. Computational[27] and experimental studies[23] have shown that a similar LYVE1^{pos} macrophage cluster is present in many tissues, with a conserved role in regulating blood vessel stiffness and permeability[23,28]. Mechanistically, the LYVE1^{pos} cluster can produce IL-10, transforming growth factor-β (TGFβ) family members and matrix metalloproteinase 9 (MMP-9), which together control inflammation and regulate the dynamics of collagen deposition during resolution of inflammation[23,28]. The experimental deletion of LYVE1^{pos} macrophages in the lung leads to increased leukocyte infiltration and excessive fibrosis[23], whereas a lack of these cells around the arterial wall leads to increased arterial stiffness owing to greater deposition of collagen by smooth muscle cells[28]. In the synovium, LYVE1^{pos} macrophages are not only located in close proximity to blood vessels but also interact directly with CD90^{pos} (also known as THY1^{pos}) sublining synovial fibroblasts[8] that provide the extracellular matrix that supports the structure of the synovium[16], suggesting a similar role for LYVE1^{pos} macrophages in regulating interstitial extracellular matrix deposition. Many regulatory functions of this cluster, such as extracellular matrix homeostasis and vessel mechanics, are mediated by the interaction of LYVE1 with its ligand hyaluronic acid, which is expressed on recipient stromal cells (such as smooth muscle cells)[28,29]. In summary, LYVE1^{pos} STMs likely control synovial blood vessel permeability and blood cell infiltration within the tissue and regulate tissue remodelling.

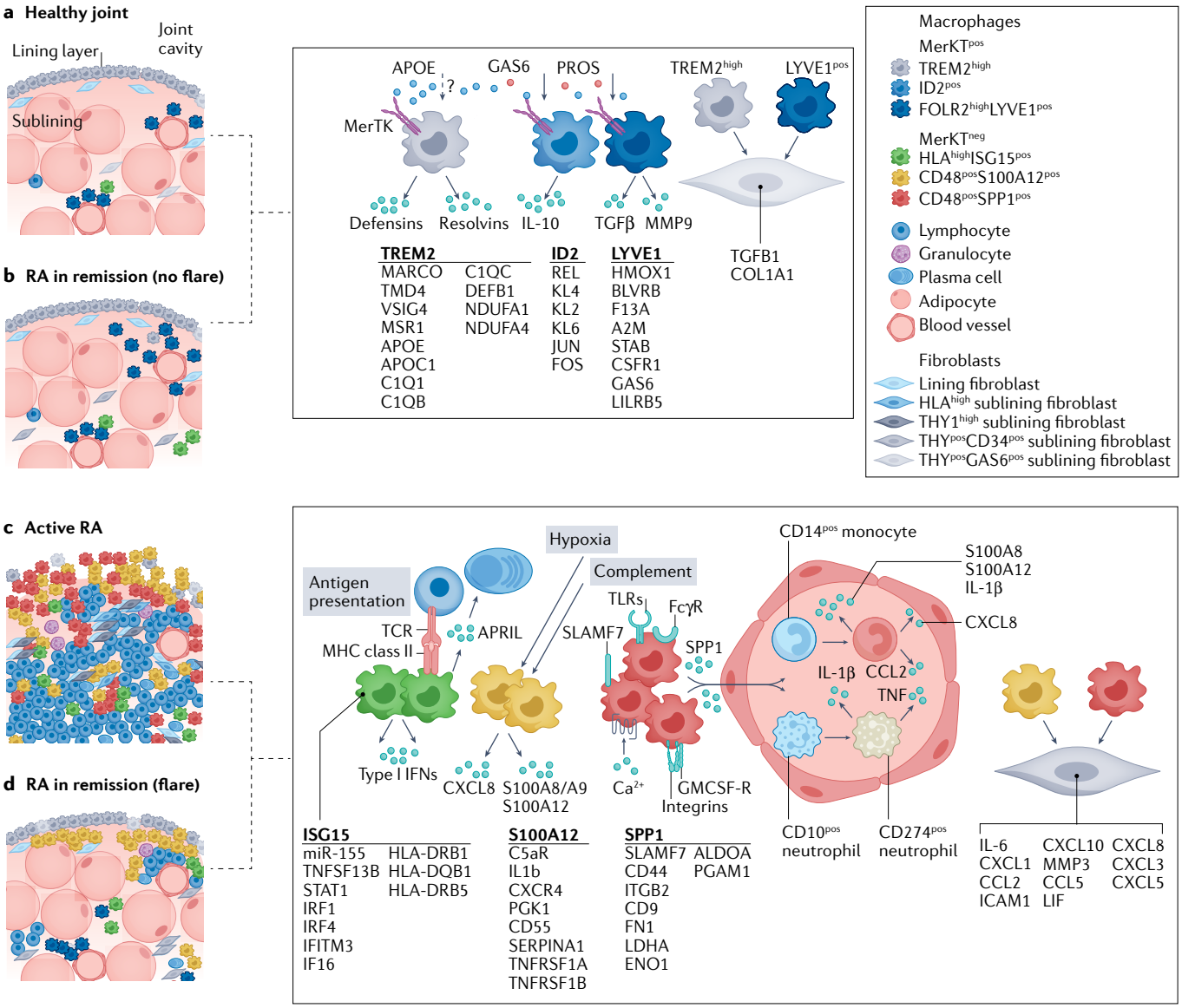
Initiation of inflammation in the synovium. Studies in patients, as well as experimental animal models, have revealed dynamic STM behaviour during the very early stages of arthritis (that is, undifferentiated arthritis)[8,9,21]. These works indicate that a phenotypic switch in the tissue-resident lining-layer (TREM2^{pos}) STM population precedes the influx of both neutrophils and monocyte precursors of tissue-infiltrating, inflammatory macrophage clusters (CD206^{neg}MerTK^{neg}), which are the main producers of TNF in chronic stages of arthritis[8,9,21]. Initial mouse studies showed that MHCII^{neg} tissue-resident STMs (close equivalents of human TREM2^{pos}) are crucial in limiting the inflammatory response in the joint[10]. Subsequent studies confirmed the key role of an intact TREM2^{pos} STM lining layer barrier in controlling inflammation. Indeed, diphtheria toxin-induced depletion of TREM2^{pos} lining-layer macrophages (that is, TREM2^{pos} STM expressing diphtheria toxin receptor under the CX3CR1 promoter) or pharmacological disintegration of lining layer tight junctions resulted in early and accelerated neutrophil influx that exacerbated the onset and severity of arthritis in experimental models of

Box 1 | **The function of MerTK in tissue resident macrophages**

MerTK is a protein tyrosine kinase receptor that belongs to the TAM family receptors (Tyro3, AXL, MerTK). MerTK is mainly expressed by tissue-resident macrophages (MAC) and mediates the immuno-homeostatic functions of these cells. Activation of MerTK by one of the two ligands: growth-arrest-specific 6 (GAS6) or protein S (PROS), promotes apoptotic cell removal[103,104], resolution of tissue inflammation[105,106], tissue remodelling[104] and tissue spatial organization[107].



- Tissue homeostasis (apoptotic cells removal)
- Resolution of tissue inflammation
- Tissue remodelling
- Tissue spatial organization


a Healthy joint
Lining layer
Joint cavity
Sublining
b RA in remission (no flare)
c Active RA
d RA in remission (flare)
APOE
GAS6
PROS
TREM2high
LYVE1pos
MerTK
Defensins
Resolvins
IL-10
TGFβ
MMP9
TGFB1
COL1A1
TREM2
MARCO
TMD4
VSIG4
MSR1
APOE
APOC1
C1Q1
C1QB
C1QC
DEFB1
NDUFA1
NDUFA4
ID2
REL
KL4
KL2
KL6
JUN
FOS
LYVE1
HMOX1
BLVRB
F13A
A2M
STAB
CSFR1
GAS6
LILRB5
Macrophages
MerKTpos
TREM2high
ID2pos
FOLR2highLYVE1pos
MerKTneg
HLAhighISG15pos
CD48posS100A12pos
CD48posSPP1pos
Lymphocyte
Granulocyte
Plasma cell
Adipocyte
Blood vessel
Fibroblasts
Lining fibroblast
HLAhigh sublining fibroblast
THY1high sublining fibroblast
THYposCD34pos sublining fibroblast
THYposGAS6pos sublining fibroblast
Hypoxia
Complement
Antigen presentation
TCR
MHC class II
APRIL
SLAMF7
TLRs
FcγR
SPP1
Type I IFNs
CXCL8
S100A8/A9
S100A12
Ca2+
GMCSF-R
Integrins
CD14pos monocyte
S100A8
S100A12
IL-1β
CXCL8
IL-1β
CCL2
TNF
CD10pos neutrophil
CD274pos neutrophil
IL-6
CXCL10
CXCL8
CXCL1
MMP3
CXCL3
CCL2
CCL5
CXCL5
ICAM1
LIF
ISG15
miR-155
TNFSF13B
STAT1
IRF1
IRF4
IFITM3
IF16
HLA-DRB1
HLA-DQB1
HLA-DRB5
S100A12
C5aR
IL1b
CXCR4
PGK1
CD55
SERPINA1
TNFRSF1A
TNFRSF1B
SPP1
SLAMF7
CD44
ITGB2
CD9
FN1
LDHA
ENO1
ALDOA
PGAM1

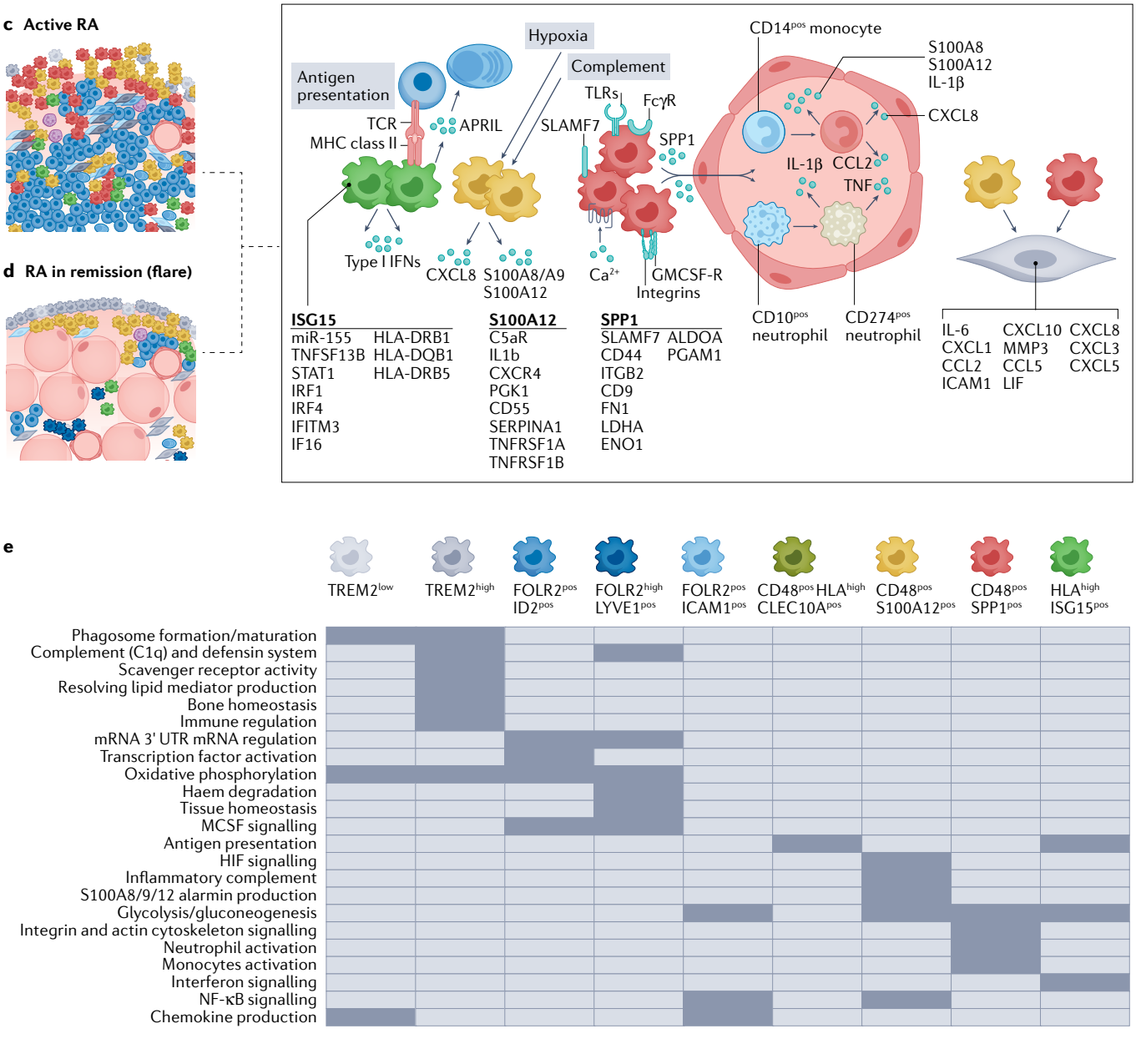

e
TREM2low
TREM2high
FOLR2pos ID2pos
FOLR2high LYVE1pos
FOLR2pos ICAM1pos
CD48pos HLAhigh CLEC10Apos
CD48pos S100A12pos
CD48pos SPP1pos
HLAhigh ISG15pos
Phagosome formation/maturation
Complement (C1q) and defensin system
Scavenger receptor activity
Resolving lipid mediator production
Bone homeostasis
Immune regulation
mRNA 3' UTR mRNA regulation
Transcription factor activation
Oxidative phosphorylation
Haem degradation
Tissue homeostasis
MCSF signalling
Antigen presentation
HIF signalling
Inflammatory complement
S100A8/9/12 alarmin production
Glycolysis/gluconeogenesis
Integrin and actin cytoskeleton signalling
Neutrophil activation
Monocytes activation
Interferon signalling
NF-κB signalling
Chemokine production

Fig. 2 | **Diverse functions of STM clusters in health, inflammation and remission in RA. a** | The healthy synovial membrane is a pauci-cellular tissue that covers the inner surface of the joint cavity. It contains tissue-resident MerTK^{pos}CD206^{pos} synovial tissue macrophages (STMs) with two main clusters: TREM2^{pos}, which forms a barrier layer, and LYVE1^{pos}, which is mostly localized in the sublining layer. **b** | In patients with rheumatoid arthritis (RA) in sustained clinical and ultrasound remission without flare, the STM composition is similar to that of the healthy joint with a restoration of MerTK^{pos}CD206^{pos} clusters, whereby TREM2^{pos} and LYVE1^{pos} clusters actively promote the resolution of inflammation by releasing resolvins and anti-inflammatory mediators (such as IL-10, defensins and TGFβ). These tissue-resident STMs induce an anti-inflammatory and a repair programme (characterized by TGFB1, COL1A1, COL1A1.1 and CD24) in stromal cells, contributing to maintaining an inflammation-free joint environment. GAS6, PROS and APOE represent potential drivers of the phenotypes of MerTK^{pos}CD206^{pos} clusters. **c** | The synovial membrane in active RA is characterized by progressive changes in the synovial lining and sublining layers, as well as infiltration of immune cells that leads to synovial hyperplasia. These tissue alterations are associated with changes in the composition and phenotype of STMs. Synovial inflammation leads to breakdown of the epithelial-like barrier and change in the phenotype of lining-layer STMs from TREM2^{high} to TREM2^{low} with reduced regulatory pathway function and increased expression of chemokines. These events contribute to the development of a permissive synovial tissue niche that fosters the influx and differentiation of monocyte-derived tissue-infiltrating (MerTK^{neg}CD206^{neg}) STMs (CD48^{pos}S100A12^{pos}, CD48^{pos}SPP1^{pos} and CD48^{pos}HLA^{pos}ISG15^{pos}) that amplify synovitis through the production of pro-inflammatory mediators, activation of stromal cells and promotion of T and B lymphocyte activation. For example, these tissue-infiltrating MerTK^{neg}CD206^{neg} STMs induce chemokines, cytokines and MMP production by synovial fibroblasts. **d** | The synovium of remission patients with RA who flare is characterized by the persistence of the CD48^{pos}S100A12^{pos} STM cluster, which produces alarmins to rekindle inflammation. **e** | Clusters constituting the main human MerTK^{neg}CD206^{neg} and MerTK^{pos}CD206^{pos} STM populations differ in metabolic and immune pathways, suggesting cluster-specific functions (generated from information in Alivernini et al.[8] and Clayton et al.[102]).

antibody-transfer RA. Human studies on early arthritis showed that disruption to this lining-layer barrier can be preceded by an increase in the numbers of lining layer macrophages[30] and their differentiation into a TREM2^{low} cluster[8]. The TREM2^{low} cluster has decreased expression of many regulatory pathways, such as VSIG4 and retinoic acid pathways and increased expression of inflammatory mediators such as S100A9, IL-8 and SPP1, compared with the mother TREM2^{pos} cluster[8]. The switch from a TREM2^{pos} into a TREM2^{low} cluster was most prominent in very early arthritis[8] and mouse studies suggest that immune complexes containing autoantibodies might be responsible for this phenotypic change[9]. In humans, such differentiation likely permits the development of inflammation in response to anti-citrullinated protein autoantibodies (ACPA) or endogenous TLR ligands. In summary, TREM2^{pos} STMs control the onset of inflammation, and changes in their phenotype towards TREM2^{low} might be the first step in the initiation of synovitis, such as by producing mediators attracting neutrophils (for example, IL-8) and monocytes (for example, SPP1).

Perpetuated joint inflammation. Clinical arthritis is associated with hypertrophy of the synovial membrane, which reflects a number of alterations to the synovium, including hyperplasia of the inner lining and sublining layers, fibroblast expansion, increased blood and lymphatic vasculature and an influx of immune cells[31]. In at least two of the most common types of synovitis — myeloid (macrophage rich) and lymphoid (T and B cell follicle rich) — inflammatory macrophages (CD206^{neg}MerTK^{neg}) are the key cellular component[31,32]. Notably, this population is different from tissue-resident (CD206^{pos}MerTK^{pos}) STMs that are present in healthy synovium and likely differentiates locally from circulating monocytes that are attracted to the synovium by chemokines[33]. Indeed, recent studies have provided evidence of accelerated monocytopoiesis in bone marrow, and the egress of immature CD14^{pos} precursors from bone marrow and their migration into inflamed joints[34]. In vivo tracking of labelled blood CD14^{pos} cells from patients with RA confirmed that CD14^{pos} cells migrate to the joints[35,36]. Whether other monocyte subpopulations, such as CD16^{pos} and CD14^{pos}CD16^{pos} cells, are also trafficked into and within human synovial tissue is unclear. However, in line with this possibility, mouse studies have revealed that Ly6C^{pos} classical monocytes (equivalent of human CD14^{pos} monocytes) and Ly6C^{neg} patrolling monocytes (equivalent of human CD16^{pos} and CD14^{pos}CD16^{pos} monocytes) differentiate into pro-inflammatory macrophages that mediated joint pathology[10,37].

Tissue-infiltrating (CD206^{neg}MerTK^{neg}) STMs are abundant macrophage population in the synovium of patients with active RA, including in patients who are naive to treatment or resistant to conventional DMARDs. Consistent with their pro-inflammatory function, an ex vivo study of synovial biopsy-derived, FACS-sorted tissue-infiltrating (CD206^{neg}MerTK^{neg}) STMs showed that these cells are the main producers of pro-inflammatory cytokines (such as TNF, IL-6 and IL-1β) and chemokines (such as CCL2)[8]. This fact is in sharp contrast to tissue-resident (CD206^{pos}MerTK^{pos}) STMs, which show limited production of these inflammatory mediators in accordance with their more homeostatic and regulatory role[8].

In addition to the production of pro-inflammatory cytokines and chemokines, each of the three identified clusters that comprise the tissue-infiltrating (CD206^{neg}MerTK^{neg}) STM population seem to have their own unique pathogenic mediator signature, suggesting discrete mechanistic contributions to synovitis. First, the CD48^{pos}S100A12^{pos} cluster shows the highest expression levels of inflammation-triggering alarmins (such as S100A8, S100A9 and S100A12) and CXCL8 (REF.[8]), which are potent inducers of monocyte and fibroblast activation and chemo-attractants for neutrophils, respectively. This cluster also seems to be the major source of IL-1β in the inflamed synovium[7,8]. Second, the CD48^{pos}SPP1^{pos} cluster is characterized by high levels of glycolytic enzymes, cytoskeletal proteins and integrins, suggesting an activated migratory phenotype. Moreover, its marker SPP1 (also known as osteopontin) has bone-resorbing properties, indicating a specific contribution of this cluster to bone damage[38]. SPP1 also drives differentiation of the pro-inflammatory neutrophil cluster (PDL1^{pos})[39], suggesting that this STM cluster might also contribute to the activation of neutrophils attracted to the synovial fluid. It was shown recently that their strong pro-inflammatory activation, including TNF production, is driven by the engagement of signalling lymphocytic activation molecule family member 7 (SLAMF7)[40]. Finally, the transcriptome of the third tissue-infiltrating cluster (CD48^{pos}ISG15^{pos}) suggests that this population is a source of type I IFNs in the synovium and therefore

Monocytopoiesis
A process that leads to the differentiation of monocytes from hematopoietic precursors in the bone marrow.

might contribute to type I interferon-driven pathological conditions observed in some patients with a poor response to therapies[41]. Of the three clusters, this one also expresses the highest level of the plasma cell survival factor APRIL, suggesting that it has a role in ectopic germinal centre formation. A recent study showed that their pro-inflammatory activation is likely driven by the post-transcriptional regulator, microRNA-155 (REF.[42]) that limits the cellular pool of inhibitors of inflammatory responses, such as SOCS-1 and SHIP1 proteins[43]. While all three clusters are present in patients with active RA, SPP1^{pos} and ISG15^{pos} clusters are further increased in patients that are resistant to conventional DMARDs treatment[8,42]. Together, these findings highlight that each of the three tissue-infiltrating (CD206^{neg}MerTK^{neg}) STM subpopulations might make unique contributions towards synovitis. Nonetheless, although these STM clusters are all localized in the sublining layer, their specific local pathogenic micro-niches (such as perivascular, ectopic germinal centre or enrichment in adipocytes) have yet to be identified. Understanding the precise niche in which these STM populations exert biological effects is important to better understand the contribution of macrophage diversity to different pathogenic features of synovitis.

The mechanism of tissue-infiltrating (CD206^{neg}MerTK^{neg}) STM-mediated inflammation involves activation of synovial fibroblasts. Micro-co-cultures of synovial biopsy-derived tissue-infiltrating STMs with synovial tissue stromal cells demonstrated that this population, but not tissue-resident (CD206^{pos}MerTK^{pos}) STM clusters, triggered MMP production from lining layer fibroblasts, and pro-inflammatory cytokines and chemokines from sublining layer synovial fibroblasts[8,44]. In addition, in vitro modelling of the CD48^{pos}S100A12^{pos} cluster of tissue-infiltrating STMs (the approximate equivalent of the M1 population[7]) showed that this cluster produces heparin-binding EGF-like growth factor (HBEGF) that, in turn, promotes epidermal growth factor receptor (EGFR)-dependent invasiveness of synovial fibroblasts[44]. Together, these data suggest a key role of tissue-infiltrating (CD206^{neg}MerTK^{neg}) STMs in driving the pathogenic response of synovial fibroblasts. Interestingly, an elegant study in mice showed that inflammation-imprinted lining (PDPN^{pos}CD90^{neg}) and sublining (PDPN^{pos}CD90^{pos}) fibroblasts could perpetuate their respective pathogenic functions (joint damage or inflammation) after adoptive transfer to healthy joints[17]. Thus, reciprocal interactions between tissue-infiltrating (CD206^{neg}MerTK^{neg}) STM clusters and synovial fibroblasts might perpetuate chronic synovitis. The role of tissue-infiltrating (CD206^{neg}MerTK^{neg}) STM clusters in regulating synovial tissue effector T cell responses (such as the recently identified PD1^{high} CXCR5^{neg} helper T cells[45]) is unclear; however, their high expression of MHCII and their production of a wide range of mediators suggests that they direct the adaptive immune response. Several studies show that monocytes from the synovial fluid of patients with RA can drive the differentiation of T helper 1 and T helper 17 cells, supporting this hypothesis[46]. In summary, tissue-infiltrating STM clusters are a major source of a wide range of inflammatory mediators and trigger and imprint chronic activation of the synovial stromal compartment in RA.

Disease remission. Remission of RA is defined by the resolution of systemic and joint inflammation[47]. At a minimum, this includes restoration of the disease activity score 28 (DAS28) to less than 2.6 (REF.[47]) and resolution of synovial inflammation as confirmed by normal synovial blood flow on Power Doppler ultrasound[15,48]. Note that patients who meet these criteria for remission could still have active synovitis[49] and that more stringent criteria, such as the Boolean-based definition, have been suggested. Clinically, remission is a key part of the recovery process from RA, leading to improved joint function via restoration of homeostatic turnover of bone and reduced loss of cartilage[48,50,51]. Although sustained drug-free remission is rare, it is not impossible; thus, understanding the homeostatic mechanisms of sustained remission might encourage new therapeutic strategies.

Recent studies in patients with arthritis, as well as experimental arthritic models, suggest that STMs, particularly the tissue-resident (CD206^{pos}MerTK^{pos}) population, have a pivotal role in RA disease remission[8–10]. The synovium of patients with RA in remission shows a substantially reduced number of pathogenic tissue-infiltrating (CD206^{neg}MerTK^{neg}) STMs that are hugely abundant in active RA, and restoration of the healthy tissue-resident (CD206^{pos}MerTK^{pos}) STM clusters TREM2^{pos} and LYVE1^{pos} in the lining and sublining layers, respectively[6,8]. Investigations into the biology of remission-related tissue-resident (CD206^{pos}MerTK^{pos}) STM clusters have demonstrated their role in limiting inflammation and re-instating synovial homeostasis. This STM population produces inflammation-resolving mediators (such as Resolvin D1), rather than pro-inflammatory mediators, and induces a repair programme in synovial fibroblasts, such as increased expression of collagens and the TGFβ pathway[8]. It follows, therefore, that global deletion of tissue-resident STMs in mice during the peak of inflammation delays the resolution of experimental arthritis[10]. In summary, tissue-resident (CD206^{pos}MerTK^{pos}) STM clusters might be actively involved in the resolution of synovial inflammation and contribute to maintenance of remission.

Flare of arthritis. Of those patients with RA who respond to treatment and achieve remission, approximately half will relapse within months of treatment cessation[48,52]. A recent study revealed that the presence of tissue-infiltrating (CD206^{neg}MerTK^{neg}) STMs prior to treatment cessation, and specifically the CD48^{pos}S100A12^{pos}cluster, as identified by scRNAseq, is associated with an increased risk of subsequent flare[8]. Isolation of these STMs from biopsy material of patients with RA in sustained clinical and ultrasound remission, revealed high expression levels of inflammation-triggering alarmins, such as S100A8, S100A9 and S100A12, similar to levels expressed by equivalent CD48^{pos}S100A12^{pos} clusters isolated from patients with active RA. These findings suggest that the inflammatory activities of the CD48^{pos}S100A12^{pos}

Boolean
The ACR/EULAR definition of remission by which, at any time point, a patient must satisfy all of the following: painful joint ≤1, swollen joint ≤1, C-reactive protein ≤1 mg/dl and Patient Global Assessment ≤1 (on a 0–10 scale).

Epigenetic imprinting
Changes in the chromatin structure around a specific gene that is induced by the environment and that makes a gene primed for either higher or lower expression levels. These changes can be passed from mother to daughter cell.

STM clusters might be responsible for triggering flares of arthritis if not controlled by the treatment and/or the regulatory functions of CD206^{pos}MerTK^{pos} STMs. Other cell types likely also contribute to disease flares. An elegant study recently identified circulating stromal cells in the blood of patients with RA prior to flare, with a CD45^{neg}CD31^{neg}PDPN^{pos} phenotype that resembled synovial sublining-layer fibroblasts[53]. Uncovering whether there is a functional and temporal link between the breakdown of the synovial lining layer barrier, emergence of the CD48^{pos}S100A12^{pos} STM cluster and the appearance of CD45^{neg}CD31^{neg}PDPN^{pos} fibroblasts in the circulation prior to flare, would help to establish a cellular interactome that might be prognostic or targeted therapeutically to improve patient management.

Sustained remission versus healthy homeostasis. The structure and histology of the RA synovium in sustained remission can resemble the healthy synovium, with both containing similar inflammation-controlling STM clusters. However, even prolonged remission eventually flares[54,55], demonstrating that remission is fragile and far from a complete return to health. Thus, STM phenotypes in the context of remission require closer scrutiny to better understand the pathological mechanisms. Detailed molecular studies of patients with RA in remission have shown that, compared with healthy controls, inner lining TREM2^{pos} and sublining LYVE1^{pos} STM clusters fail to restore the expression of certain regulatory pathways that are downregulated in active RA[8]. The results suggest that these 'super-repressed' genes might contribute to the tendency for flare-ups in drug-free RA remission. For example, STMs from patients with RA in remission remain deficient in *VSIG4*, which encodes a B7-related co-inhibitory molecule[1], in *LILRB* genes, which encode inhibitors of Fc gamma receptor-driven activation[56], and in genes encoding enzymes involved in retinoic acid production (such as *ALDH1A1* and *RBP4*)[8]. The role of these molecules in STM-driven joint homeostasis is unknown, although their known functions in other contexts suggest a role in restricting adaptive immunity[57]. For example, the expression of VSIG4 in myeloid cells inhibits effector T cell function and activates FOXP3^{pos} regulatory T cells[32]. Moreover, global *Vsig4*$^{-/-}$ mice spontaneously develop inflammatory and autoimmune pathological conditions[58], whereas a VSIG4-Fc fusion-protein protects against experimental arthritis[59].

The mechanisms that sustain the super-repressed gene expression pattern in remission are unknown. Possible candidates include the action of autoantibodies (that is, the breach of self-tolerance) and/or epigenetic changes in STM precursors. Serological evidence of remission, defined as the disappearance of a previously positive test for anti-citrullinated protein antibody (ACPA) and/or rheumatoid factor after successful therapy, is a rare event in patients with RA. It is noteworthy, though, that specific B cell targeted therapies, such as rituximab and CTLA4-Ig, can decrease autoantibody titres[60–62]. In support of the notion that autoantibodies might be responsible for the different signatures of STMs from healthy individuals and from patients with RA in remission, recent studies have demonstrated that ACPA positivity is associated with a higher probability of disease flare after treatment modification[63,64]. An additional, but not mutually exclusive, possibility is that tissue-resident (MerTK^{pos}CD206^{pos}) STMs and their local precursors undergo epigenetic imprinting mediated by prior inflammation. Indeed, fate-mapping studies of murine counterparts of both TREM2^{pos} and LYVE1^{pos} populations suggest that tissue-resident STMs can be long-lived[9,10], rendering them susceptible to epigenetic imprinting by the local environment. A better understanding of the difference in myeloid pathways between remission and healthy synovium will help to develop new treatment strategies to extend remission towards restoration of self-sustained, normal joint homeostasis.

Specification of distinct STM clusters

Tissue macrophages undergo dramatic and diverse responses to danger signals, including infection, aberrant immunity and tissue injury. This development of distinct immune-homeostatic functions in response to such signals might be determined by at least two factors: their ontogeny and adaptation to tissue-specific cues.

Ontogeny

Murine models have established that adult healthy tissues contain resident macrophages that can originate from three different sources: embryonic yolk sac precursors, such as microglia; fetal liver precursors, such as alveolar macrophages; and adult bone marrow-derived monocytes, such as the majority of macrophages in the gut and dermis[65]. Yolk sac and liver prenatal precursors are established in tissues during early embryonic development and macrophage numbers are maintained in adulthood by in situ proliferation. In certain tissues, such as the lung, macrophage numbers can be supplemented postnatally by recruiting blood monocytes. In other tissues, such as the gut, the tissue-resident macrophages are constantly replenished by recruited blood monocytes[66,67].

In addition to the initial source of macrophages, the tissue-resident macrophage population is enriched by monocyte-derived macrophages during infection or injury, which respond to locally generated cytokine danger signals[66]. Recent studies in mice suggest that TREM2^{pos} synovial lining-layer tissue-resident macrophages are of prenatal origin and maintained by precursors proliferating in situ[9,10]. First, bone marrow transplant studies showed that synovial tissue-resident MHCII^{neg} (equivalent of TREM2^{pos} STMs) were long-lived, with their tissue pool not requiring a contribution from bone marrow for at least 2 months post-transplant[10]. This finding was supported by data showing that TREM2^{pos} macrophages are present in the synovium by embryonic day 15.5, suggesting that TREM2^{pos} STMs are derived from embryonic precursors[9]. Using a cell fate-mapping approach, it was demonstrated that the half-life of mouse TREM2^{pos} STMs is approximately 5 weeks, and that these cells are maintained in adulthood by M-CSFR^{pos} precursors proliferating in situ and independent of precursors from the circulation[9]. These mouse studies indicate a similar

embryonic origin for the synovial tissue RELMa^{pos} population (human LYVE1^{pos})[9]. It is still unclear whether human TREM2^{pos} and LYVE1^{pos} STMs clusters similarly differentiate from prenatal precursors that proliferate in situ, or whether these clusters are maintained by recruited circulating monocytes. A small population of LYVE1^{pos}ID2^{pos} STMs, closely resembling mouse M-CSFR^{pos} precursors, have been identified in healthy and arthritic human synovium[8], providing indirect evidence to support the prenatal precursor hypothesis. Human tissue-resident TREM2^{pos}, LYVE1^{pos} and LYVE1^{low}ID2^{pos} STM clusters express high levels of FOLR2, a protein that mediates the delivery of folic acid derivatives into the cell. Given that derivatives of folic acid are key for DNA synthesis, DNA repair, maintaining epigenetic marks and adhesion to collagen, the folic acid–folate receptor 2 (FOLR2) pathway might contribute to the fitness and longevity of these tissue-resident STM clusters[68–71].

Uncovering the origin of human tissue-resident STMs might improve our understanding of the mechanisms that underpin the deficiency in several regulatory pathways that persists in patients with RA, and in disease remission, such as that seen in TREM2^{pos} and LYVE1^{pos} STM clusters. Mouse studies suggest that monocyte-derived macrophages that adopt a tissue-resident phenotype might have different, perhaps impaired functions compared with the original resident macrophage population. For example, experimental repopulation of the lung alveolar niche with monocyte-derived macrophages impairs lung immune homeostasis[67,72]. This scenario resembles the changes in alveolar macrophages that occur with advanced age or lung injury, in which fetal liver-derived homeostatic alveolar macrophages are replaced by inflammatory monocyte-derived alveolar macrophages. Although the monocyte-derived alveolar macrophages can efficiently recycle pulmonary surfactant to enable efficient gas exchange[72], they have a more pro-fibrotic[73] and pro-inflammatory (as potent producers of IL-6)[74] phenotype than the original resident alveolar macrophages. Thus, a similar replacement of initial TREM2^{pos} macrophages of prenatal origin with inflammation-directed monocyte-derived macrophages in the synovium of patients with RA might contribute to the suboptimal joint immune homoeostasis in disease remission. Although emerging evidence suggests a prenatal origin of tissue-resident (MerTK^{pos}CD206^{pos}) STM clusters, experimental data suggest that pro-inflammatory tissue-infiltrating (MerTK^{neg}CD206^{neg}) STM clusters differentiate from infiltrating blood precursors. Human studies with radiolabelled CD14^{pos} monocytes[36], as well as mouse bone-marrow transplant and cell fate-tracking studies[9,10], strongly suggest that during arthritis both the CD14^{pos}CD16^{neg} and CD14^{pos}CD16^{pos} populations of blood monocytes give rise to the inflammatory macrophages, although it is unclear which monocyte population gives rise to which MerTK^{neg}CD206^{neg} STM clusters. In mice, depletion of blood monocytes using an anti-CCR2 antibody, or replacement of CCR2 competent (wild type) bone marrow with *Ccr2*$^{-/-}$ bone-marrow transplant, prevented the development of the mouse equivalent of human tissue-infiltrating (MerTK^{neg}) CD48^{pos}S100A12^{pos} and CD48^{pos}ISG15^{pos} STM clusters in the synovium that was associated with attenuated joint swelling in a mouse model of arthritis[10]. In summary, to better understand the origin of different human STMs, a close tracking of STM populations in patients administered autologous radiolabelled precursors, or monitoring of patients with RA after bone-marrow transplant, is required.

Tissue-specific cues

All tissue macrophage precursors acquire their identity via the action of the lineage-determining transcription factor PU.1, which itself is transcriptionally controlled by stromal compartment-derived M-CSF and IL-34 (REF.[1]). Subsequently, tissue-specific identity is provided by tissue-specific cues that induce differentiation of macrophages towards tissue-specific functions, such as bone remodelling osteoclasts, surfactant-recycling alveolar macrophages or iron-recycling red zone macrophages in the spleen[67]. The identity of 'tissue-identity' signals for many types of tissue macrophages have been uncovered, although the signals determining the identity of the lining-layer TREM2^{pos} STMs and sublining layer LYVE1^{pos} STMs, remain elusive.

In the lung, epithelial cell-derived granulocyte–macrophage colony-stimulating factor (GM–CSF) and surfactants act through transcription factors PPARγ[75] and BACH2 (REF.[76]) to induce the unique identity of alveolar macrophages, generating a cell capable of recycling surfactants to maintain patency and facilitate gas exchange[77]. Mice and humans lacking functional alveolar macrophages develop severe lung proteinosis owing to uncontrolled accumulation of surfactants, demonstrating the importance of this macrophage population for tissue homeostasis. Intriguingly, human TREM2^{pos} lining-layer STMs share transcriptomic similarities with human alveolar macrophages that also express TREM2 (REF.[39]), potentially reflecting similar biological functions. One possibility is that TREM2^{pos} STMs recycle components of synovial fluid, such as lubricin, which is produced by PDPN^{pos}PRG4^{pos} lining-layer fibroblasts to facilitate joint movement[78]. Thus, by analogy with the lung, lining-layer fibroblast-derived lubricin might drive the protective, inflammation-resolving barrier functions of the lining-layer.

Transcription factors that are specific to the lining (TREM2^{pos}) STM cluster have not yet been identified, but FLIP (FLICE-like inhibitory protein) might, at least in part, be responsible for their differentiation and inflammation-resolving phenotypes, including their expression of VSIG4, a receptor with immunosuppressive function[21]. Similarly, the drivers and transcription factors of the sub-lining (LYVE1^{pos}) cluster are unknown. Making some progress in this area, a recent study demonstrated that endothelial cell-derived JAG1 acts via its receptor NOTCH3 to determine the transcriptomic identity of the sublining synovial fibroblast clusters (CD90^{pos})[22]. A similar pathway might be involved in the function of the LYVE1^{pos} STM cluster that localizes around blood vessels in the joint and might regulate influx of inflammatory cells. Studies of STMs

that have transcriptional similarities to those in other tissues in homeostasis will likely provide insight into additional regulators of their functions. These might include the haem-driven transcription factor NRF2 (REF.[26]) or endothelial cell-derived R-spondin 3 (an activator of the canonical WNT signalling pathway)[79], both of which regulate the function of blood vessel-associated macrophages in liver and lung. Identification of STM-identity cues could help to design therapeutic strategies to boost their numbers or homeostatic functions in patients with RA.

Response to emerging danger signals

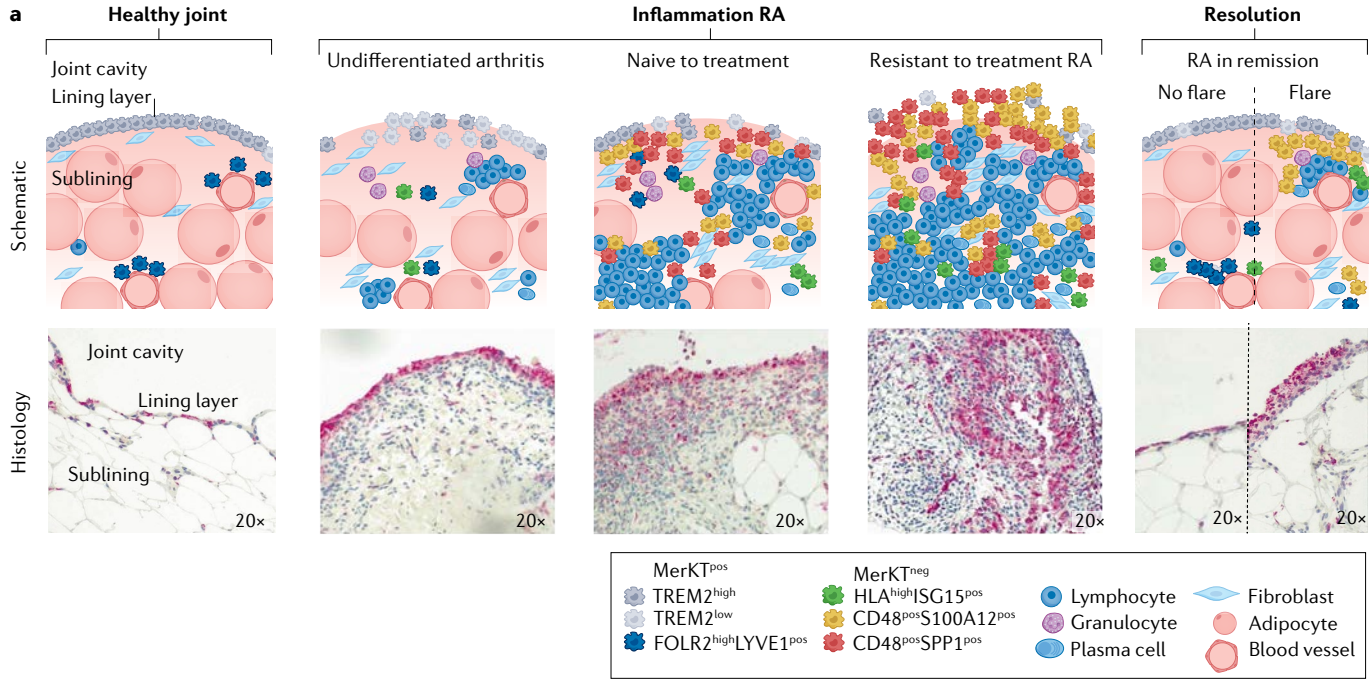
Tissue-resident and infiltrating monocyte-derived macrophages can shape the tissue environment in response to emerging 'immediate demand' signals, such as during infection or injury. During the development of synovitis, a variety of signals are thought to drive the pro-inflammatory activation of tissue-infiltrating (MerTK^{neg}CD206^{neg}) STMs, including pro-inflammatory cytokines, endogenous TLR ligands, oxidized lipids, interactions with epigenetically imprinted synovial fibroblasts and T cells (reviewed in REF.[80]). However, the precise upstream regulators of individual tissue-infiltrating STM clusters remain unknown. A comparison of scRNAseq datasets from human macrophage clusters in a range of inflamed tissues, including synovium, identified two inflammatory clusters that are shared between tissues: CXCL10- and CCL2-expressing macrophages and FCN1^{pos} macrophages[81], which are the equivalents of CD48^{pos}SPP1^{pos} and CD48^{pos}S100A12^{pos}[8,39] STM clusters, respectively. Emergence of these clusters in multiple tissues during inflammation suggests that they share a common upstream regulator. A preliminary computational prediction, supported by in vitro data, suggests that a combination of TNF plus IFNγ might be responsible for the differentiation of, at least, the CXCL10 and CCL2 cluster[81] (CD48^{pos}SPP1^{pos}[8,39]), but in vivo confirmation is needed. Recent studies suggest that high extracellular calcium levels can also drive differentiation of the CD48^{pos}SPP1^{pos} macrophage cluster from monocyte precursors, and that monocytes from patients with RA more easily respond to the calcium signal[82]. High levels of calcium are present in synovial fluid of patients with RA[83] and may be responsible for the differentiation of joint infiltrating monocytes into CD48^{pos}SPP1^{pos} pro-inflammatory macrophages in the RA synovium.

Compared with tissue-infiltrating (MerTK^{neg}CD206^{neg}) STMs, tissue-resident (MerTK^{pos}CD206^{pos}) clusters seem to respond differently to inflammatory signals, involving engagement of the GAS6–MerTK pathway to resolve inflammation. GAS6 is expressed by sublining synovial fibroblast clusters[7,8] and this expression is increased in synovial fibroblasts of patients in RA disease remission[8]. GAS6–MerTK interactions drive the expression of transcription factors (including KLF4 and NR4A2) that govern the active inflammation-resolving phenotypes of both MerTK^{pos}TREM2^{pos} and MerTK^{pos}LYVE1^{pos} STM clusters[84,85]. More detailed studies are required to confirm how the molecular interplay between the tissue-resident STMs and the tissue-infiltrating STMs determine chronicity or resolution of tissue inflammation.

STMs as treatment response targets in RA

Sustained disease remission is currently the target clinical outcome in RA management[86]. Although progress has been made in predicting the response to different therapies, the goal of personalized medicine, enabling identification of the most effective drug to induce early remission for a particular patient, is still elusive. Many of the therapeutics approved for the treatment of RA inhibit the inflammatory function of macrophages. In addition, early studies showed that macrophages can be biomarkers of a good therapeutic response, and therefore useful predictors of the response to some RA treatments. For example, an effective treatment response to gold, sulphasalazine, methotrexate or leflunomide was associated with a reduced macrophage infiltration into synovial sublining tissue following treatment[87]. STMs isolated from patients with RA undergoing synovectomy showed that leflunomide and methotrexate inhibit the release of pro-inflammatory cytokines and NF-κB expression[88]. Similarly, treatments targeting TNF (such as etanercept and infliximab) are associated with a reduction in the number of, and upregulated apoptotic signal in, STMs[89]. However, most mechanistic data investigating the therapeutic effect of various recent biological treatments on macrophage functions are derived from in vitro studies on monocyte-derived macrophages from the blood of patients with RA or from the assessment of mouse models of arthritis. Although these studies are informative[90], they provide limited insight into the changes in the composition of STM clusters and their resolving functions. For example, although in RA the main anti-inflammatory mechanism of methotrexate is the modulation of adenosine metabolism[91], methotrexate is also a folate antagonist with low binding affinity to FOLR2 (REF.[92]), a receptor that is highly expressed on tissue-resident TREM2^{pos} and LYVE1^{pos} STMs. Even though patients with RA are given supplementary folic acid with methotrexate, the role of methotrexate in modulating tissue-resident lining layer and perivascular STM functions are unknown. Thus, understanding the impact of therapeutics on various STM clusters, and the consequence on the synovial environment, requires synovial biopsy-driven investigations.

The development of minimally invasive biopsy techniques has enabled synovial tissue collection for high-throughput analysis and markedly improved our understanding of RA heterogeneity and response to therapies. The synovial biopsy-based study of the Pathobiology Early Arthritis Cohort (PEAC) established three different synovial pathotypes: 'diffuse myeloid', characterized by monocyte or macrophage enrichment; 'lympho-myeloid', characterized by aggregates of B and T lymphocytes with a variable degree of inflammatory cell infiltrate; and 'pauci-immune fibroid', characterized by a lack of an inflammatory cell infiltrate. RA consists predominantly (~85–90%) of the 'diffuse myeloid' and 'lympho-myeloid' pathotypes, and macrophages are the commonest histopathological feature regardless of lymphocyte enrichment[93]. The degree of enrichment of STMs can predict an effective response to TNF inhibition, compared with the poor response in patients with RA with a pauci-immune synovial pathotype[94].



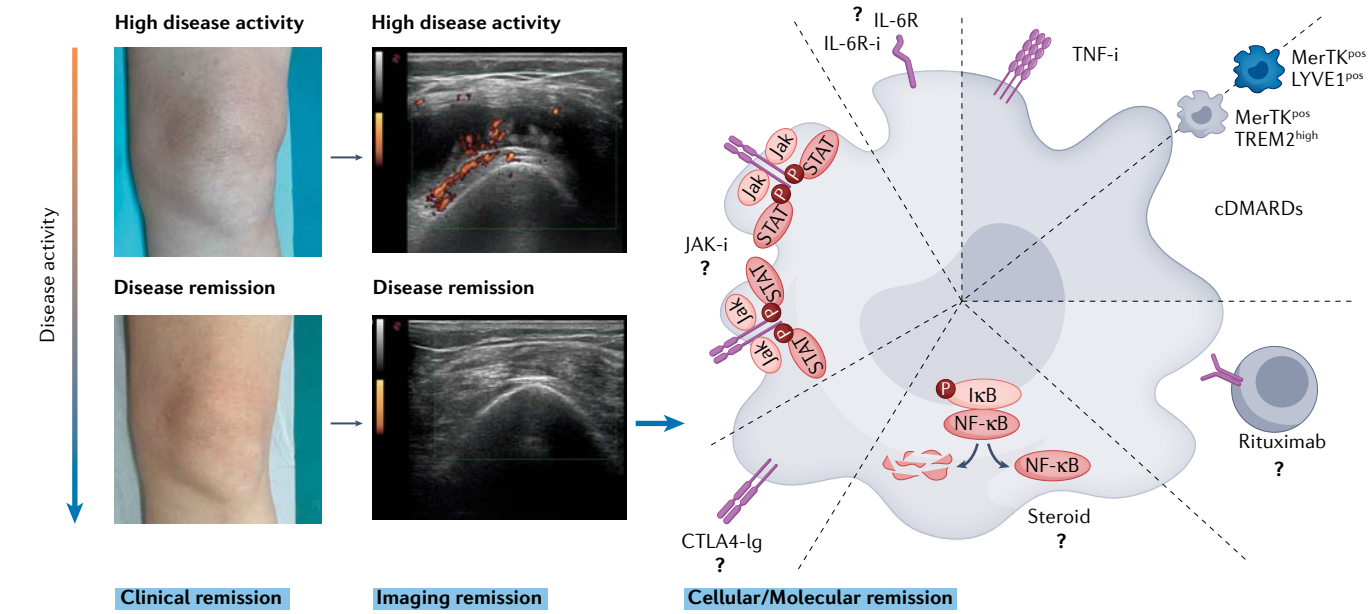


Fig. 3 | **STM clusters as novel biomarkers of treatment-response in RA.** **a** | Top: schematic showing changes in synovial tissue macrophage (STM) composition during RA progression. In RA, barrier-forming TREM2^{pos} switch to an inflammation-permitting TREM2^{low} phenotype in very early undifferentiated arthritis. In RA that is naive to treatment, an increase in the number of sublining MerTK^{neg}CD206^{neg} clusters S100A12^{pos}, SPP1^{pos} and ISG15^{pos} is observed. A further increase in SPP1^{pos} and ISG15^{pos} clusters is seen in patients with RA that are resistant to conventional DMARDs. Restoration of healthy STM composition, including the lining layer TREM2^{pos} barrier cluster and sublining LYVE1^{pos} cluster, occurs in patients in remission RA who do not flare after treatment tapering/cessation, whereas patients who flare exhibit the emergence of the pro-inflammatory MerTK^{neg}CD206^{neg} S100A12^{pos} cluster in the sublining layer. Bottom: representative histology images of staining for pan macrophage marker CD68 (red) on ultrasound-guided minimally invasive synovial tissue biopsies are shown at different RA stages. **b** | Levels of RA remission. The current definition of remission includes clinically based (such as DAS28- or Boolean-based) and imaging-based (such as ultrasonography) parameters. Representative images of the knee joint from a patient with active RA and from a patient with RA in sustained clinical remission (left) and their ultrasound Power Doppler evaluation (right) are shown. These illustrate an increased blood flow associated with inflammation (positive Power Doppler signal) in the knee of a patient with active RA and a lack of Power Doppler signal in the knee of a patient in remission, confirming a lack of inflammation. Patient knee images, ultrasonography and histology images come from the SYNGem Biopsy Unit (Fondazione Policlinico Universitario A. Gemelli IRCCS, Rome, Italy). Emerging studies indicate that inclusion of a cellular and molecular description of STMs can enhance the power of algorithms designed to predict sustained disease remission in patients with RA. For example, patients who achieved disease remission with methotrexate and TNF inhibitors (TNF-i) have a reduced risk of disease flare after treatment cessation if their synovium is enriched in the MerTK^{pos}CD206^{pos} STM clusters, TREM2^{pos} and LYVE1^{pos}. The phenotype and molecular signature of STMs during sustained clinical and imaging remission achieved with other treatments (such as IL-6R inhibition (IL-6Ri), JAK inhibition (JAKi), CTLA4-Ig, steroids and rituximab) are unknown.

Omics
Genomics, proteomics, metabolomics and transcriptomics aimed at the collective characterization and quantification of pools of biological molecules revealing the biology of the cells.

In addition, synovial tissue expression of gene modules characteristic of pro-inflammatory macrophages correlated with clinical parameters such as disease activity measured with DAS28-CRP[95]. This finding is in line with those of earlier studies showing that synovial tissue enriched with macrophages expressing CD163, MRP14 (now called S100A9) and MRP8 (now called S100A8) that produced TNF in situ predicted a good response to TNF inhibition[96]. Similarly, comparison of anti-IL-6R with TNF-inhibitor therapy (the ADACTA trial) defined that a baseline myeloid synovitis was associated with a good clinical response at 24 weeks of TNF inhibitor treatment[97]. By contrast, patients with RA who responded inadequately to TNF inhibition, and who were classified as having a B cell poor synovitis (defined histologically and by RNA sequencing) had more successful treatment responses to IL-6R inhibition compared with TNF or B cell-targeted therapy[98].

Preliminary studies suggest that the macrophage cytokine profile released by synovial explant in vitro can predict response to RA therapies. For example, the concentration of inflammatory mediators, such as IL-6, MCP-1 and TNF, were associated with disease activity and structural characteristics of the source joint[99]; high production of IL-6 and MCP-1 correlated with the subsequent response to TNF inhibitiors[100]. Thus, pro-inflammatory macrophage-rich synovitis is a useful biomarker for predicting therapeutic responses, at least to TNF inhibitors.

Recent analysis of prospective longitudinal synovial samples from patients with RA suggests a continuum in synovial pathotypes from a pauci-immune, fibroid pathotype to a lympho-myeloid pathotype, with progressive involvement of innate and adaptive immune cells leading to the formation of ectopic lymphoid structures with plasma cells[95,101]. In this inflammatory spectrum, infiltrating macrophages that function as a source of pro-inflammatory cytokines and antigen-presenting cells to T follicular helper cells might be fundamental for local B cell activation and maturation of plasma cells. Therefore, in this context, despite pathotypes representing different types of synovitis characterized by different pathogenetic and inflammatory milieu, the heterogeneous functions of STMs could represent evolving states of activation in the disease process[95]. Thus, identifying specific STM clusters associated with progression between pathotypes will help us to understand the cellular and molecular mechanisms that underlie this transition and provide potential biomarkers to predict the evolution of the disease in terms of response to specific treatments.

Patients with RA who achieve sustained disease remission might experience disease flare immediately after tapering or cessation of treatment, with approximately half of patients developing a flare within a year[15]. Typically, the duration of drug-free remission is limited[54], with a progressive increase in the risk of disease flare over time[55]. Thus, identifying patients at risk is the first step in preventing the occurrence of flare, although prediction methods are lacking. A recent study showed that in patients who achieved sustained remission (defined by clinical and ultrasound criteria) with anti-TNF plus methotrexate treatment, the relative proportions of the main STM populations (that is, tissue-resident regulatory MerTK^{pos}CD206^{pos} versus tissue-infiltrating, pro-inflammatory MerTK^{neg}CD206^{neg}) at the time of treatment tapering or cessation can predict whether the patient remains in remission for at least a year[8]. This finding suggests that identifying the phenotypes of STMs might provide insight into the underlying mechanisms of remission and serve as a useful predictor of sustained disease remission versus disease flare. Similar studies are required to investigate the composition and phenotypes of STMs in patients with RA in remission following different treatments (FIG. 3). Recently, an increasing number of algorithms have been developed to predict the response to treatments, such as by quantifying the nature and extent of synovial tissue inflammation, tissue pathotypes and transcriptomics, duration of symptoms, autoantibody positivity and the response to first-line treatment[30,95]. Emerging evidence presented in this Review suggests that recognition of the phenotype and function of the STM clusters, as well as their relative proportions, could strengthen the predictive power of these models.

Conclusions

Recent advances in single-cell omics have uncovered a rich heterogeneity in STMs. The discovery of distinct STM clusters that are vital for joint homeostasis and maintaining disease remission provides a paradigm shift in our understanding of the role of macrophages in arthritis. Capitalizing on the joint protective and inflammation-resolving biology of newly identified STM clusters might aid the development of novel therapeutics that combat arthritis and maintain disease remission. In addition, emerging data suggest that profiling STM clusters could clarify the mechanisms of disease progression and help to predict response to treatment in patients with RA and other chronic inflammatory joint disorders.

Published online 7 June 2022

1. Okabe, Y. & Medzhitov, R. Tissue biology perspective on macrophages. *Nat. Immunol.* **17**, 9–17 (2016).
2. Watanabe, S., Alexander, M., Misharin, A. V. & Budinger, G. R. S. The role of macrophages in the resolution of inflammation. *J. Clin. Invest.* **129**, 2619–2628 (2019).
3. Smolen, J. S. et al. Rheumatoid arthritis. *Nat. Rev. Dis. Prim.* **4**, 18001 (2018).
4. Mulherin, D., Fitzgerald, O. & Bresnihan, B. Synovial tissue macrophage populations and articular damage in rheumatoid arthritis. *Arthritis Rheumatol.* **39**, 115–124 (1996).
5. Tak, P. P. et al. Analysis of the synovial cell infiltrate in early rheumatoid synovial tissue in relation to local disease activity. *Arthritis Rheumatol.* **40**, 217–225 (1997).
6. Schett, G., McInnes, I. B. & Neurath, M. F. Reframing immune-mediated inflammatory diseases through signature cytokine hubs. *N. Engl. J. Med.* **385**, 628–639 (2021).
7. Zhang, F. et al. Defining inflammatory cell states in rheumatoid arthritis joint synovial tissues by integrating single-cell transcriptomics and mass cytometry. *Nat. Immunol.* **20**, 928–942 (2019).
8. Alivernini, S. et al. Distinct synovial tissue macrophage subsets regulate inflammation and remission in rheumatoid arthritis. *Nat. Med.* **26**, 1295–1306 (2020).
9. Culemann, S. et al. Locally renewing resident synovial macrophages provide a protective barrier for the joint. *Nature* https://doi.org/10.1038/s41586-019-1471-1 (2019).
10. Misharin, A. V. et al. Nonclassical Ly6C$^-$ monocytes drive the development of inflammatory arthritis in mice. *Cell Rep.* **9**, 591–604 (2014).
11. Lemke, G. & Rothlin, C. V. Immunobiology of the TAM receptors. *Nat. Rev. Immunol.* **8**, 327–336 (2008).
12. Gordon, S. Alternative activation of macrophages. *Nat. Rev. Immunol.* **3**, 23–35 (2003).
13. Wood, M. J. et al. Macrophage proliferation distinguishes 2 subgroups of knee osteoarthritis

patients. *JCI Insight* https://doi.org/10.1172/jci.insight.125325 (2019).
14. Yager, N. et al. Ex vivo mass cytometry analysis reveals a profound myeloid proinflammatory signature in psoriatic arthritis synovial fluid. *Ann. Rheum. Dis.* https://doi.org/10.1136/annrheumdis-2021-220280 (2021).
15. Alivernini, S. et al. Tapering and discontinuation of TNF-alpha blockers without disease relapse using ultrasonography as a tool to identify patients with rheumatoid arthritis in clinical and histological remission. *Arthritis Res. Ther.* **18**, 39 (2016).
16. Firestein, G. S., Budd, R. C., Gabriel, S. E., McInnes, I. B. & O'Dell, J. R. *Kelley and Firestein's textbook of rheumatology*. 10th edn. (Elsevier Health Sciences, 2016).
17. Croft, A. P. et al. Distinct fibroblast subsets drive inflammation and damage in arthritis. *Nature* **570**, 246–251 (2019).
18. Smith, I. D. et al. Rapid *in situ* chondrocyte death induced by *Staphylococcus aureus* toxins in a bovine cartilage explant model of septic arthritis. *Osteoarthritis Cartilage* **21**, 1755–1765 (2013).
19. Gautier, E. L. et al. Gene-expression profiles and transcriptional regulatory pathways that underlie the identity and diversity of mouse tissue macrophages. *Nat. Immunol.* **13**, 1118–1128 (2012).
20. Jaitin, D. A. et al. Lipid-associated macrophages control metabolic homeostasis in a Trem2-dependent manner. *Cell* **178**, 686–698.e14 (2019).
21. Huang, Q. Q. et al. Critical role of synovial tissue-resident macrophage niche in joint homeostasis and suppression of chronic inflammation. *Sci. Adv.* https://doi.org/10.1126/sciadv.abd0515 (2021).
22. Wei, K. et al. Notch signalling drives synovial fibroblast identity and arthritis pathology. *Nature* **582**, 259–264 (2020).
23. Chakarov, S. et al. Two distinct interstitial macrophage populations coexist across tissues in specific subtissular niches. *Science* https://doi.org/10.1126/science.aau0964 (2019).
24. Ural, B. B. et al. Identification of a nerve-associated, lung-resident interstitial macrophage subset with distinct localization and immunoregulatory properties. *Sci. Immunol.* https://doi.org/10.1126/sciimmunol.aax8756 (2020).
25. Muller, P. A. et al. Crosstalk between muscularis macrophages and enteric neurons regulates gastrointestinal motility. *Cell* **158**, 300–313 (2014).
26. Pfefferle, M. et al. Hemolysis transforms liver macrophages into antiinflammatory erythrophagocytes. *J. Clin. Invest.* **130**, 5576–5590 (2020).
27. Mulder, K. et al. Cross-tissue single-cell landscape of human monocytes and macrophages in health and disease. *Immunity* **54**, 1883–1900.e5 (2021).
28. Lim, H. Y. et al. Hyaluronan receptor LYVE-1-expressing macrophages maintain arterial tone through hyaluronan-mediated regulation of smooth muscle cell collagen. *Immunity* **49**, 326–341.e7 (2018).
29. Lawrance, W., Banerji, S., Day, A. J., Bhattacharjee, S. & Jackson, D. G. Binding of hyaluronan to the native lymphatic vessel endothelial receptor LYVE-1 is critically dependent on receptor clustering and hyaluronan organization. *J. Biol. Chem.* **291**, 8014–8030 (2016).
30. Alivernini, S. et al. Inclusion of synovial tissue-derived characteristics in a nomogram for the prediction of treatment response in treatment-naive rheumatoid arthritis patients. *Arthritis Rheumatol.* **73**, 1601–1613 (2021).
31. Firestein, G. S. & McInnes, I. B. Immunopathogenesis of rheumatoid arthritis. *Immunity* **46**, 183–196 (2017).
32. Pitzalis, C., Kelly, S. & Humby, F. New learnings on the pathophysiology of RA from synovial biopsies. *Curr. Opin. Rheumatol.* **25**, 334–344 (2013).
33. Yeo, L. et al. Expression of chemokines CXCL4 and CXCL7 by synovial macrophages defines an early stage of rheumatoid arthritis. *Ann. Rheum. Dis.* **75**, 763–771 (2016).
34. Smiljanovic, B. et al. Monocyte alterations in rheumatoid arthritis are dominated by preterm release from bone marrow and prominent triggering in the joint. *Ann. Rheum. Dis.* **77**, 300–308 (2018).
35. Thurlings, R. M. et al. Monocyte scintigraphy in rheumatoid arthritis: the dynamics of monocyte migration in immune-mediated inflammatory disease. *PLoS ONE* **4**, e7865 (2009).
36. Herenius, M. M. et al. Monocyte migration to the synovium in rheumatoid arthritis patients treated with adalimumab. *Ann. Rheum. Dis.* **70**, 1160–1162 (2011).
37. Weiss, M. et al. IRF5 controls both acute and chronic inflammation. *Proc. Natl Acad. Sci. USA* **112**, 11001–11006 (2015).
38. Kahles, F., Findeisen, H. M. & Bruemmer, D. Osteopontin: a novel regulator at the cross roads of inflammation, obesity and diabetes. *Mol. Metab.* **3**, 384–393 (2014).
39. MacDonald, L. et al. COVID-19 and RA share an SPP1 myeloid pathway that drives PD-L1^{+} neutrophils and CD14^{+} monocytes. *JCI Insight* https://doi.org/10.1172/jci.insight.147413 (2021).
40. Simmons, D. P. et al. SLAMF7 engagement superactivates macrophages in acute and chronic inflammation. *Sci. Immunol.* **7**, eabf2846 (2022).
41. Cooles, F. A. H. et al. The interferon gene signature is increased in patients with early treatment-naive rheumatoid arthritis and predicts a poorer response to initial therapy. *J. Allergy Clin. Immunol.* **141**, 445–448 e444 (2018).
42. Olsson, A. M. et al. miR-155-overexpressing monocytes resemble HLA^{high}ISG15^{+} synovial tissue macrophages from patients with rheumatoid arthritis and induce polyfunctional CD4^{+} T-cell activation. *Clin. Exp. Immunol.* **207**, 188–198 (2022).
43. Kurowska-Stolarska, M. et al. MicroRNA-155 as a proinflammatory regulator in clinical and experimental arthritis. *Proc. Natl Acad. Sci. USA* **108**, 11193–11198 (2011).
44. Kuo, D. et al. HBEGF^{+} macrophages in rheumatoid arthritis induce fibroblast invasiveness. *Sci. Transl. Med.* https://doi.org/10.1126/scitranslmed.aau8587 (2019).
45. Rao, D. A. et al. Pathologically expanded peripheral T helper cell subset drives B cells in rheumatoid arthritis. *Nature* **542**, 110–114 (2017).
46. Roberts, C. A., Dickinson, A. K. & Taams, L. S. The interplay between monocytes/macrophages and CD4^{+} T cell subsets in rheumatoid arthritis. *Front. Immunol.* **6**, 571 (2015).
47. van der Heijde, D. et al. Comparison of different definitions to classify remission and sustained remission: 1 year TEMPO results. *Ann. Rheum. Dis.* **64**, 1582–1587 (2005).
48. Alivernini, S. et al. Synovial features of patients with rheumatoid arthritis and psoriatic arthritis in clinical and ultrasound remission differ under anti-TNF therapy: a clue to interpret different chances of relapse after clinical remission? *Ann. Rheum. Dis.* https://doi.org/10.1136/annrheumdis-2016-210424 (2017).
49. Makinen, H., Kautiainen, H., Hannonen, P. & Sokka, T. Is DAS28 an appropriate tool to assess remission in rheumatoid arthritis? *Ann. Rheum. Dis.* **64**, 1410–1413 (2005).
50. Kawashiri, S. Y. et al. Ultrasound-detected bone erosion is a relapse risk factor after discontinuation of biologic disease-modifying antirheumatic drugs in patients with rheumatoid arthritis whose ultrasound power Doppler synovitis activity and clinical disease activity are well controlled. *Arthritis Res. Ther.* **19**, 108 (2017).
51. Dirven, L. et al. Changes in hand bone mineral density and the association with the level of disease activity in patients with rheumatoid arthritis: bone mineral density measurements in a multicenter randomized clinical trial. *Arthritis Care Res.* **63**, 1691–1699 (2011).
52. Nagy, G. & van Vollenhoven, R. F. Sustained biologic-free and drug-free remission in rheumatoid arthritis, where are we now? *Arthritis Res. Ther.* **17**, 181 (2015).
53. Orange, D. E. et al. RNA identification of PRIME cells predicting rheumatoid arthritis flares. *N. Engl. J. Med.* **383**, 218–228 (2020).
54. Ajeganova, S. & Huizinga, T. Sustained remission in rheumatoid arthritis: latest evidence and clinical considerations. *Ther. Adv. Musculoskelet. Dis.* **9**, 249–262 (2017).
55. Heimans, L. et al. Two-year results of disease activity score (DAS)-remission-steered treatment strategies aiming at drug-free remission in early arthritis patients (the IMPROVED-study). *Arthritis Res. Ther.* **18**, 23 (2016).
56. van der Touw, W., Chen, H. M., Pan, P. Y. & Chen, S. H. LILRB receptor-mediated regulation of myeloid cell maturation and function. *Cancer Immunol. Immunother.* **66**, 1079–1087 (2017).
57. Yuan, X., Yang, B. H., Dong, Y., Yamamura, A. & Fu, W. CRIg, a tissue-resident macrophage specific immune checkpoint molecule, promotes immunological tolerance in NOD mice, via a dual role in effector and regulatory T cells. *Elife* https://doi.org/10.7554/eLife.29540 (2017).
58. Li, J. et al. VSIG4 inhibits proinflammatory macrophage activation by reprogramming mitochondrial pyruvate metabolism. *Nat. Commun.* **8**, 1322 (2017).
59. Katschke, K. J. Jr et al. A novel inhibitor of the alternative pathway of complement reverses inflammation and bone destruction in experimental arthritis. *J. Exp. Med.* **204**, 1319–1325 (2007).
60. de Moel, E. C. et al. In RA, becoming seronegative over the first year of treatment does not translate to better chances of drug-free remission. *Ann. Rheum. Dis.* **77**, 1836–1838 (2018).
61. Jansen, D. et al. Conversion to seronegative status after abatacept treatment in patients with early and poor prognostic rheumatoid arthritis is associated with better radiographic outcomes and sustained remission: post hoc analysis of the AGREE study. *RMD Open* **4**, e000564 (2018).
62. Bozec, A. et al. Abatacept blocks anti-citrullinated protein antibody and rheumatoid factor mediated cytokine production in human macrophages in IDO-dependent manner. *Arthritis Res. Ther.* **20**, 24 (2018).
63. Haschka, J. et al. Relapse rates in patients with rheumatoid arthritis in stable remission tapering or stopping antirheumatic therapy: interim results from the prospective randomised controlled RETRO study. *Ann. Rheum. Dis.* **75**, 45–51 (2016).
64. Rech, J. et al. Prediction of disease relapses by multibiomarker disease activity and autoantibody status in patients with rheumatoid arthritis on tapering DMARD treatment. *Ann. Rheum. Dis.* **75**, 1637–1644 (2016).
65. Varol, C., Mildner, A. & Jung, S. Macrophages: development and tissue specialization. *Annu. Rev. Immunol.* **33**, 643–675 (2015).
66. Cox, N., Pokrovskii, M., Vicario, R. & Geissmann, F. Origins, biology, and diseases of tissue macrophages. *Annu. Rev. Immunol.* **39**, 313–344 (2021).
67. Ginhoux, F. & Guilliams, M. Tissue-resident macrophage ontogeny and homeostasis. *Immunity* **44**, 439–449 (2016).
68. Samaniego, R. et al. Macrophage uptake and accumulation of folates are polarization-dependent in vitro and in vivo and are regulated by activin A. *J. Leukoc. Biol.* **95**, 797–808 (2014).
69. Xia, W. et al. A functional folate receptor is induced during macrophage activation and can be used to target drugs to activated macrophages. *Blood* **113**, 438–446 (2009).
70. Turk, M. J. et al. Folate-targeted imaging of activated macrophages in rats with adjuvant-induced arthritis. *Arthritis Rheum.* **46**, 1947–1955 (2002).
71. Machacek, C. et al. Folate receptor beta regulates integrin CD11b/CD18 adhesion of a macrophage subset to collagen. *J. Immunol.* **197**, 2229–2238 (2016).
72. van de Laar, L. et al. Yolk sac macrophages, fetal liver, and adult monocytes can colonize an empty niche and develop into functional tissue-resident macrophages. *Immunity* **44**, 755–768 (2016).
73. Misharin, A. V. et al. Monocyte-derived alveolar macrophages drive lung fibrosis and persist in the lung over the life span. *J. Exp. Med.* **214**, 2387–2404 (2017).
74. Aegerter, H. et al. Influenza-induced monocyte-derived alveolar macrophages confer prolonged antibacterial protection. *Nat. Immunol.* **21**, 145–157 (2020).
75. Schneider, C. et al. Induction of the nuclear receptor PPAR-gamma by the cytokine GM-CSF is critical for the differentiation of fetal monocytes into alveolar macrophages. *Nat. Immunol.* **15**, 1026–1037 (2014).
76. Nakamura, A. et al. Transcription repressor Bach2 is required for pulmonary surfactant homeostasis and alveolar macrophage function. *J. Exp. Med.* **210**, 2191–2204 (2013).
77. Morales-Nebreda, L., Misharin, A. V., Perlman, H. & Budinger, G. R. The heterogeneity of lung macrophages in the susceptibility to disease. *Eur. Respir. Rev.* **24**, 505–509 (2015).
78. Kurowska-Stolarska, M. & Alivernini, S. Synovial tissue macrophages: friend or foe? *RMD Open* **3**, e000527 (2017).
79. Zhou, B. et al. The angiocrine Rspondin3 instructs interstitial macrophage transition via metabolic-epigenetic reprogramming and resolves inflammatory injury. *Nat. Immunol.* **21**, 1430–1443 (2020).
80. Alivernini, S. et al. Driving chronicity in rheumatoid arthritis: perpetuating role of myeloid cells. *Clin. Exp. Immunol.* **193**, 13–23 (2018).
81. Zhang, F. et al. IFN-gamma and TNF-alpha drive a *CXCL10*+ *CCL2*+ macrophage phenotype expanded in severe COVID-19 lungs and inflammatory diseases

with tissue inflammation. *Genome Med.* **13**, 64 (2021).
82. Murthy, S. et al. Danger signal extracellular calcium initiates differentiation of monocytes into SPP1/osteopontin-producing macrophages. *Cell Death Dis.* **13**, 53 (2022).
83. Jager, E. et al. Calcium-sensing receptor-mediated NLRP3 inflammasome response to calciprotein particles drives inflammation in rheumatoid arthritis. *Nat. Commun.* **11**, 4243 (2020).
84. Roberts, A. W. et al. Tissue-resident macrophages are locally programmed for silent clearance of apoptotic cells. *Immunity* **47**, 913–927.e6 (2017).
85. Mahajan, S. et al. Nuclear receptor Nr4a2 promotes alternative polarization of macrophages and confers protection in sepsis. *J. Biol. Chem.* **290**, 18304–18314 (2015).
86. Smolen, J. S. et al. EULAR recommendations for the management of rheumatoid arthritis with synthetic and biological disease-modifying antirheumatic drugs: 2019 update. *Ann. Rheum. Dis.* **79**, 685–699 (2020).
87. Haringman, J. J. et al. Synovial tissue macrophages: a sensitive biomarker for response to treatment in patients with rheumatoid arthritis. *Ann. Rheum. Dis.* **64**, 834–838 (2005).
88. Cutolo, M. et al. Anti-inflammatory effects of leflunomide in combination with methotrexate on co-culture of T lymphocytes and synovial macrophages from rheumatoid arthritis patients. *Ann. Rheum. Dis.* **65**, 728–735 (2006).
89. Catrina, A. I. et al. Evidence that anti-tumor necrosis factor therapy with both etanercept and infliximab induces apoptosis in macrophages, but not lymphocytes, in rheumatoid arthritis joints: extended report. *Arthritis Rheumatol.* **52**, 61–72 (2005).
90. Boutet, M. A. et al. Novel insights into macrophage diversity in rheumatoid arthritis synovium. *Autoimmun. Rev.* **20**, 102758 (2021).
91. Friedman, B. & Cronstein, B. Methotrexate mechanism in treatment of rheumatoid arthritis. *Jt. Bone Spine* **86**, 301–307 (2019).
92. Elnakat, H. & Ratnam, M. Distribution, functionality and gene regulation of folate receptor isoforms: implications in targeted therapy. *Adv. Drug Deliv. Rev.* **56**, 1067–1084 (2004).
93. Humby, F. et al. Synovial cellular and molecular signatures stratify clinical response to csDMARD therapy and predict radiographic progression in early rheumatoid arthritis patients. *Ann. Rheum. Dis.* **78**, 761–772 (2019).
94. Nerviani, A. et al. A pauci-immune synovial pathotype predicts inadequate response to TNFα-blockade in rheumatoid arthritis patients. *Front. Immunol.* **11**, 845 (2020).
95. Lewis, M. J. et al. Molecular portraits of early rheumatoid arthritis identify clinical and treatment response phenotypes. *Cell Rep.* **28**, 2455–2470.e5 (2019).
96. Wijbrandts, C. A. et al. The clinical response to infliximab in rheumatoid arthritis is in part dependent on pretreatment tumour necrosis factor alpha expression in the synovium. *Ann. Rheum. Dis.* **67**, 1139–1144 (2008).
97. Dennis, G. Jr et al. Synovial phenotypes in rheumatoid arthritis correlate with response to biologic therapeutics. *Arthritis Res. Ther.* **16**, R90 (2014).
98. Humby, F. et al. Rituximab versus tocilizumab in anti-TNF inadequate responder patients with rheumatoid arthritis (R4RA): 16-week outcomes of a stratified, biopsy-driven, multicentre, open-label, phase 4 randomised controlled trial. *Lancet* **397**, 305–317 (2021).
99. Andersen, M. et al. Synovial explant inflammatory mediator production corresponds to rheumatoid arthritis imaging hallmarks: a cross-sectional study. *Arthritis Res. Ther.* **16**, R107 (2014).
100. Andersen, M. et al. Association between IL-6 production in synovial explants from rheumatoid arthritis patients and clinical and imaging response to biologic treatment: a pilot study. *PLoS One* **13**, e0197001 (2018).
101. Michelutti, A. et al. B-cell subsets in the joint compartments of seropositive and seronegative rheumatoid arthritis (RA) and No-RA arthritides express memory markers and ZAP70 and characterize the aggregate pattern irrespectively of the autoantibody status. *Mol. Med.* **17**, 901–909 (2011).
102. Clayton, S. A., MacDonald, L., Kurowska-Stolarska, M. & Clark, A. R. Mitochondria as key players in the pathogenesis and treatment of rheumatoid arthritis. *Front. Immunol.* **12**, 673916 (2021).
103. Davies, L. C., Jenkins, S. J., Allen, J. E. & Taylor, P. R. Tissue-resident macrophages. *Nat. Immunol.* **14**, 986–995 (2013).
104. Bosurgi, L. et al. Macrophage function in tissue repair and remodeling requires IL-4 or IL-13 with apoptotic cells. *Science* https://doi.org/10.1126/science.aai8132 (2017).
105. Cai, B. et al. MerTK cleavage limits proresolving mediator biosynthesis and exacerbates tissue inflammation. *Proc. Natl Acad. Sci. USA* **113**, 6526–6531 (2016).
106. Triantafyllou, E. et al. MerTK expressing hepatic macrophages promote the resolution of inflammation in acute liver failure. *Gut* https://doi.org/10.1136/gutjnl-2016-313615 (2017).
107. Eom, D. S. & Parichy, D. M. A macrophage relay for long-distance signaling during postembryonic tissue remodeling. *Science* **355**, 1317–1320 (2017).

Acknowledgements
This work was supported by the Research into Inflammatory Arthritis Centre *Versus Arthritis* UK (grant no. 22072) and the *Versus Arthritis* UK project grant (no. 22253 and 22273) to M.K-S, and Linea D1 (Universitā Cattolica del Sacro Cuore, no. R4124500654) and Ricerca Finalizzata Ministero della Salute (no. GR-2018-12366992) to S.A.

Author contributions
The authors contributed equally to all aspects of the Review.

Competing interests
The authors declare no competing interests.

Peer review information
Nature Reviews Rheumatology thanks Gerhard Krönke, who co-reviewed with Katharina Knab, and A. Puig-Kröger for their contribution to the peer review of this work.

Publisher's note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

RELATED LINKS
PEAC RNA-seq data: https://peac.hpc.qmul.ac.uk
Single-cell portal: https://singlecell.broadinstitute.org/single_cell/study/SCP279/amp-phase-1
Synovial atlas of macrophages in RA: http://cellatlas.mvls.gla.ac.uk/MacrophageRA/

Cellular metabolic adaptations in rheumatoid arthritis and their therapeutic implications

Ursula Fearon[1,2]✉, *Megan M. Hanlon*[1,2], *Achilleas Floudas*[1,2] *and Douglas J. Veale*[2]

Abstract | Activation of endothelium and immune cells is fundamental to the initiation of autoimmune diseases such as rheumatoid arthritis (RA), and it results in trans-endothelial cell migration and synovial fibroblast proliferation, leading to joint destruction. In RA, the synovial microvasculature is highly dysregulated, resulting in inefficient oxygen perfusion to the synovium, which, along with the high metabolic demands of activated immune and stromal cells, leads to a profoundly hypoxic microenvironment. In inflamed joints, infiltrating immune cells and synovial resident cells have great requirements for energy and nutrients, and they adapt their metabolic profiles to generate sufficient energy to support their highly activated inflammatory states. This shift in metabolic capacity of synovial cells enables them to produce the essential building blocks to support their proliferation, activation and invasiveness. Furthermore, it results in the accumulation of metabolic intermediates and alteration of redox-sensitive pathways, affecting signalling pathways that further potentiate the inflammatory response. Importantly, the inflamed synovium is a multicellular tissue, with cells differing in their metabolic requirements depending on complex cell–cell interactions, nutrient supply, metabolic intermediates and transcriptional regulation. Therefore, understanding the complex interplay between metabolic and inflammatory pathways in synovial cells in RA will provide insight into the underlying mechanisms of disease pathogenesis.

Rheumatoid arthritis (RA) is a progressive autoimmune disease, characterized by synovial inflammation and invasion of adjacent articular cartilage and bone, leading to functional disability[1]. Although the primary trigger for RA is not known, one of the earliest events involved in synovial inflammation is neoangiogenesis (formation of new blood vessels), which facilitates immune-cell infiltration and subsequent activation of resident synovial cells, transforming the normal relatively acellular synovium into an invasive, tumour-like pannus[1–3]. In the normal synovium the vascular supply is provided by many small, stable vessels, the role of which is to enable trans-synovial exchange of molecules that nourish the joint. In RA, the architecture of synovial microvasculature is highly dysregulated[4–6], and distinct macroscopic vascular patterns are found in the inflamed joint, paralleled microscopically by an increase in the number of blood vessels, which display incomplete pericyte recruitment and differential expression of growth factors[2–6]. These blood vessels are thought to remain in a 'plastic state', primed to receive environmental cues that promote endothelial cell activation and sprouting, further facilitating immune-cell trans-endothelial migration[2–6]. This poor vascular organization prevents efficient transport of nutrients and oxygen to the joint and, coupled with the high metabolic demands of activated immune and stromal cells, leads to a hypoxic microenvironment[7,8].

The concept of altered cellular metabolism and joint hypoxia was first described in the 1970s in response to observation of an elevation of metabolite concentrations in RA synovial fluid, and of activity of the metabolic enzymes glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and lactate dehydrogenase in RA synovial cells[9,10]. Subsequent studies using proton magnetic resonance spectroscopy demonstrated higher lactate-to-glucose ratios in RA synovial fluid than in fluid from healthy individuals[11,12]; the ratios correlated with cytokine concentrations and disease activity scores. In results published in 1970, use of a Clark-type electrode demonstrated lower synovial-fluid partial pressure of oxygen (pO_2) in RA than in osteoarthritis (OA)[13]. Several studies in animal models of arthritis have demonstrated synovial pO_2 levels as low as 10 mmHg[14,15].

[1]*Molecular Rheumatology, Trinity Biomedical Sciences Institute, TCD, Dublin, Ireland.*

[2]*EULAR Centre of Excellence, Centre for Arthritis and Rheumatic Diseases, St Vincent's University Hospital, Dublin, Ireland.*

✉*e-mail: fearonu@tcd.ie*

https://doi.org/10.1038/s41584-022-00771-x

Key points

- Neoangiogenesis and synovial expansion induce a bioenergetic crisis in the inflamed joint in rheumatoid arthritis (RA).
- In RA compared with healthy tissue, synovial fibroblasts undergo a metabolic shift associated with increased invasive capacity and disease activity.
- In RA, synovial stromal–immune cell interactions lead to reciprocal metabolic changes that potentiate the inflammatory response.
- In RA, myeloid cells display metabolic abnormalities associated with increased inflammatory function.
- In RA, T cell polarization and cytokine production are linked to metabolic reprogramming, and B cells are resistant to hypoxia-induced inhibition of pro-inflammatory cytokine secretion.
- Metabolic reprogramming could represent a new therapeutic strategy for patients with RA.

Oxygen levels were also low in RA synovium undergoing repair surgery[7], and direct measurement of pO_2 levels in the synovium of patients with inflammatory arthritis demonstrated synovial oxygen levels as low as 22.5 mmHg (range 3.2–54.1 mmHg)[16], whereas levels in the normal joint are thought to be ~54 mmHg[16]. In RA, synovial-tissue oxygen levels correlate inversely with macroscopic and microscopic scores of inflammation and with disease activity[8,16]. Synovial fibroblasts from patients with RA secrete more lactate than synovial fibroblasts from patients with OA, but following co-culture with T cell-conditioned media, a similar increase in lactate production occurs in both RA and OA synovial fibroblasts[17]. In synovial fluid from patients with RA, pH correlates negatively with measurements of both lactate concentration and disease activity score in 28 joints (DAS28) calculated using C-reactive protein concentration[18], an effect that might be associated with increased expression by synovial cells of the lactate transporter MCT4, which exports lactate into the extracellular space, thereby contributing to the acidification of RA synovial fluid[19]. Elevation of lactate levels within the joint is associated with a reduction of glucose concentrations, pO_2 and pH, producing an acidic microenvironment. Synovial expression of hypoxia-inducible factor 1α (HIF1α) (the master regulator of oxygen homeostasis) is higher in patients with RA than in healthy individuals[20–23], and results from in vitro studies of loss or gain of HIF1α function demonstrate a role for this protein in the mediation of inflammation, angiogenesis, immune-cell responses and cartilage damage in RA[20–24]. Furthermore, joint hypoxia not only induces HIF1α activation, but also results in activation of and interaction between several pro-inflammatory signalling pathways implicated in RA pathogenesis, including the NF-κB, PI3K–AKT–mTOR, Janus kinase–signal transducer and activator of transcription (JAK–STAT) and Notch 1 signalling pathways[24–29]. The adverse microenvironment forces synovial cells to adapt and switch their cellular metabolism to maintain their pathogenic activated states, and these changes, in addition to accumulation of metabolic intermediates, together further potentiate synovial invasiveness within the RA joint[8,10,11,14,23,30]. The metabolic intermediates that induce inflammatory responses in the joints in RA include lactate[23], succinate[31], glutamine and itaconate[32]. However, hypoxia is not the only factor involved in the alteration of metabolic pathways in the joint, and numerous studies have characterized the effects of pro-inflammatory mediators secreted in the joint, including TNF, Toll-like receptors (TLRs), serine, IL-1β, IL-17, IL-6, IL-33 and IL-27, which induce the secretion of many metabolic intermediates that can further affect inflammatory responses in the joint[33–36]. Notably, circulating immune cells that are not exposed to hypoxic conditions have different metabolic profiles in patients with RA than in healthy individuals, suggesting that systemic inflammatory pathways independent of hypoxia also have a role in shaping the metabolic inflammatory phenotypes of immune cells and their responses[30,37,38].

In this Review we discuss the role of metabolism and its perturbations in inflammatory conditions, with specific focus on synovial tissue (the target tissue of RA), and on the roles of constituent stromal and immune cells.

Altered metabolism in RA

Evidence gathered during the past 10 years demonstrates an important role for metabolic pathways in the regulation of immune and stromal cells in joints in RA, with various studies demonstrating a shift in bioenergetic profiles from a resting regulatory phenotype to one that is highly metabolically active[3,23,39,40]. Extrinsic and intrinsic signals result in metabolic reprogramming of immune cells in RA to provide energy, biomolecules and metabolic intermediates that are required for a rapid and robust immune response[39,40]. Two of the main metabolic pathways that are utilized are glycolysis and oxidative phosphorylation[23,39,40]. Briefly, as outlined in FIG. 1, under normoxic conditions one glucose molecule enters the cell and is oxidized through a series of anaerobic reactions, generating two molecules of the three-carbon compound pyruvate, along with two molecules of ATP. In the presence of sufficient oxygen, pyruvate is then decarboxylated by pyruvate dehydrogenase, and the product enters the tricarboxylic acid (TCA) cycle in the mitochondrial matrix. The electron carriers NADH and flavin adenine dinucleotide (FADH2) are major products of the TCA cycle and transfer electrons to the electron transport chain where they pass through a series of complexes, creating an electrochemical proton gradient that produces 36 molecules of ATP per glucose molecule. In the absence of oxygen, pyruvate cannot enter the TCA cycle, and it is instead converted to lactate by lactate dehydrogenase in the cytosol, generating two ATP molecules. The pentose phosphate pathway (PPP) also branches off from the glycolytic pathway at glucose-6-phosphate and generates fructose 6-phosphate and glyceraldehyde 3-phosphate through the oxidative and non-oxidative branches of the PPP, supporting anabolic growth by promoting nucleotide synthesis[41] and de novo fatty acid synthesis[42]. Furthermore, fatty acids can then enter the oxidation pathway, where mitochondrial conversion of fatty acids to acetyl coenzyme A (acetyl-CoA), NADH and other co-factors occurs, generating additional energy for synovial cell responses (FIG. 1).

In the context of RA, PET–MRI hybrid imaging in vivo demonstrates that elevation of metabolic activity

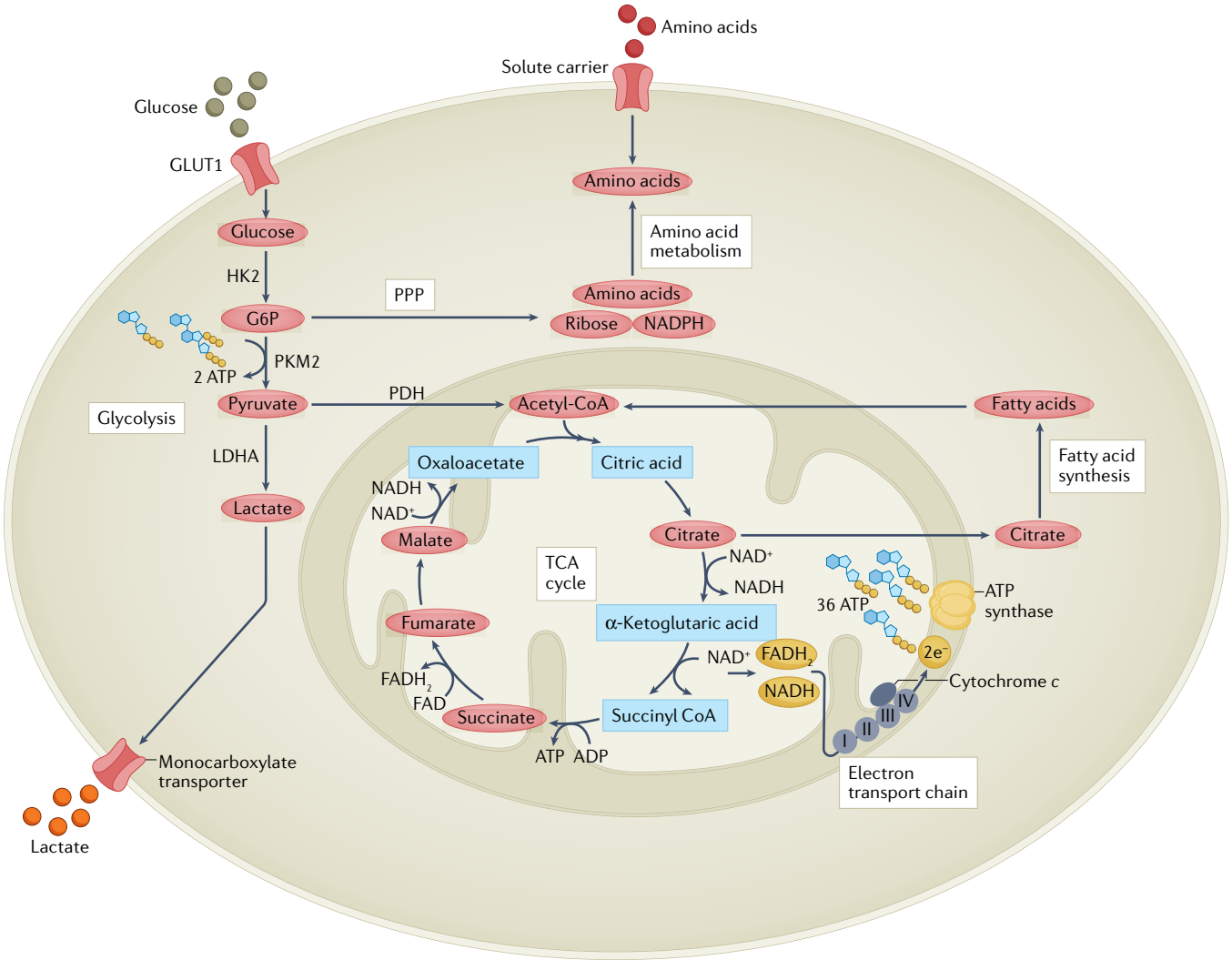


Fig. 1 | **Overview of the main cellular metabolic pathways.** Glucose enters the cell via glucose transporter 1 (GLUT1) and enters the glycolytic pathway. Hexokinase 2 (HK2) converts glucose to glucose 6-phosphate (G6P). Glycolysis generates pyruvate from glucose with the involvement of pyruvate kinase M2 (PKM2), generating the energy in the form of ATP. Pyruvate is either converted via lactate dehydrogenase (LDHA) to lactate (which is secreted), or decarboxylated by pyruvate dehydrogenase (PDH) to produce acetyl coenzyme A (acetyl-CoA), which enters the tricarboxylic acid (TCA) cycle, thereby generating NADH and flavin adenine dinucleotide ($FADH_2$) for the electron transport chain, which in turn produces 36 molecules of ATP. G6P also feeds into the pentose phosphate pathway (PPP), leading to production of ribose, NADPH and amino acids. Metabolism of amino acids can produce substrates for the TCA cycle, leading to ATP production by the electron transport chain. Citrate from the TCA cycle contributes to fatty acid synthesis, and fatty acid oxidation also feeds into the TCA cycle via the generation of acetyl-CoA.

is associated with joint inflammation[23], an effect that is reversed in patients who respond to treatment with TNF inhibitors[8,23]. Mitochondrial dysfunction[43], differential expression of glucose transporters GLUT1 and GLUT4 (REF.[44]), elevation of expression of glycolytic enzymes[3,23,45–48] and accumulation of metabolic intermediates[23,31,32] have all been observed in the RA joint, and are involved in perpetuation of the disease. Furthermore, glycolytic enzymes and products, such as enolase, aldolase and pyruvate, can act as autoantigens, stimulating abnormal cell proliferation, angiogenesis and pannus formation[31,45,46,49–51]. Finally, metabolic reprogramming using inhibitors of specific pathways or metabolic intermediates leads to resolution of inflammation in in vitro, ex vivo and in vivo models of RA[23,48,51–57]. However, the synovial microenvironment in RA is very complex, with many cell types and subtypes interacting to promote the inflammatory response, including T cells, B cells, monocytes, macrophages, dendritic cells (DCs), synovial fibroblasts and endothelial cells, which all differ in their functional roles and their utilization of metabolites. Within this adverse microenvironment, cells compete for available nutrients, and have differential responses to the metabolites produced by the various cell types within the RA joint. Therefore, it is necessary to describe what is known about the metabolic pathways in different cell types that contribute to the pathogenesis of RA.

Synovial endothelial cells and metabolism

Endothelial cells and angiogenesis. New blood-vessel formation is one of the primary events in synovial inflammation, and it facilitates the influx of immune cells into

the synovial membrane. Dysregulation of angiogenic mechanisms is an early phenomenon that is associated with the pathogenesis of RA[58]. Evidence suggests that a metabolic shift in pro-angiogenic processes is necessary to supply the required energy and biomolecule demands for new vessel formation[59]. However, the metabolic requirements of endothelial cells depend on the specific cell subtypes. The three main identified microvascular endothelial cell subsets are the tip, stalk and phalanx cells, and their metabolic profiles are determined by their specific functions (migration, proliferation and quiescence, respectively)[59] (FIG. 2). Indeed, the specialized functions of these endothelial cells are important for the development of a proper functioning vessel, which is dependent on their spatial organization within the inflamed microenvironment. New blood-vessel formation requires one endothelial cell to initially respond to a stimulus that initiates sprouting. This endothelial cell detects the highest concentration of the stimulus and becomes the tip cell, which is highly polarized and which leads the migration of endothelial cells away from their parent cells. Through lateral inhibition the selected tip cell instructs its neighbouring endothelial cells to become stalk cells, which proliferate behind the tip cell to form the growing blood vessel. Once blood flow is established, the endothelial cells revert back to a quiescent phenotype and become phalanx cells.

Endothelial cell metabolism in angiogenesis. When endothelial cells are activated, metabolic changes dictate phenotypic differentiation, with tip and stalk cells showing greater glycolytic rates than phalanx cells, which have a reliance on oxidative phosphorylation[60–62]. Despite their exposure to high oxygen concentrations, tip and stalk cells utilize anaerobic glycolysis as an energy source, enabling rapid production of ATP. The initial development of sprouts from existing blood vessels requires the guiding tip cell, which has a distinct molecular profile defined by the presence of the Notch ligand Delta-like protein 4, the surface adhesion glycoprotein CD34, the axon guidance netrin receptor UNC5B, neuropilin 1, CXCR4 and vascular endothelial growth factor (VEGF) receptors 2 and 3 (REFS[60–63]), which are expressed by RA synovial vasculature[58]. In particular, a glycolytic flux is required for the migrating tip cell to enable directed cell migration, extension and proliferation towards the angiogenic stimulus during new vessel formation[60,64]. Although metabolic regulation of synovial vessels in the inflamed joint is not well studied, macroscopic analysis through direct visualization using keyhole video arthroscopy demonstrates a dysfunctional morphology, similar to that observed in tumour vasculature[5]. pO_2 is low in the RA synovium, and inversely correlates with blood-vessel immaturity and oxidative stress[5]. Upregulation of expression of GLUT1 and key glycolytic enzymes GAPDH and pyruvate kinase M2 (PKM2) occurs in the vascular regions of the synovium in RA, and is reversed in response to treatment with TNF inhibitors[23]. Exposure of endothelial cells to hypoxia, oxidative stress and pro-inflammatory mediators induces tube formation, migration, leukocyte adhesion and invasion, and these effects are accompanied by a shift to glycolysis and activation of key transcription factors STAT3, Notch 1IC and NF-κB[23,27,65–67]. Hypoxia and synergistic interactions between VEGF and angiopoetin 2 (which are highly expressed in the synovium in RA) induce endothelial cell–cell communication through interaction between Delta-like protein 4 and Notch 1, leading to proteolytic cleavage of Notch 1IC[67]. This domain translocates to the nucleus and activates transcription of target genes involved in regulation of tip cell–stalk cell lateral inhibition, which is critical for tip-cell selection[27,67]. A key irreversible step in glycolysis is the activation of 6-phosphofructo-1-kinase, which mediates the phosphorylation of fructose 6-phosphate to fructose 1,6 bisphosphate[68]. This activation is tightly regulated by the actions of a family of 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatases. Blockade of 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3 (PFKFB3) represses unrestrained 6-phosphofructo-1-kinase-induced glycolytic flux sufficiently to reduce pathogenic pro-angiogenic activity of endothelial cells without impeding general maintenance and functionality of normal endothelial cells[69]. In RA, PFKFB3 inhibition reduces the ability of endothelial cells to form angiogenic tubes, an effect paralleled by inhibition of key signalling mediators including phosphorylated STAT3 and Notch 1IC[23]. Consistently, in human microvascular endothelial cells in vitro, PFKFB3 blockade inhibits angiogenesis induced by inhibition of the microRNA miR-125a, and in zebrafish embryos with knockout of miR-125a, PFKFB3 blockade induces normalization of vascular development[70]. Notably, miR-125a-deficient endothelial cells have higher expression of glucose

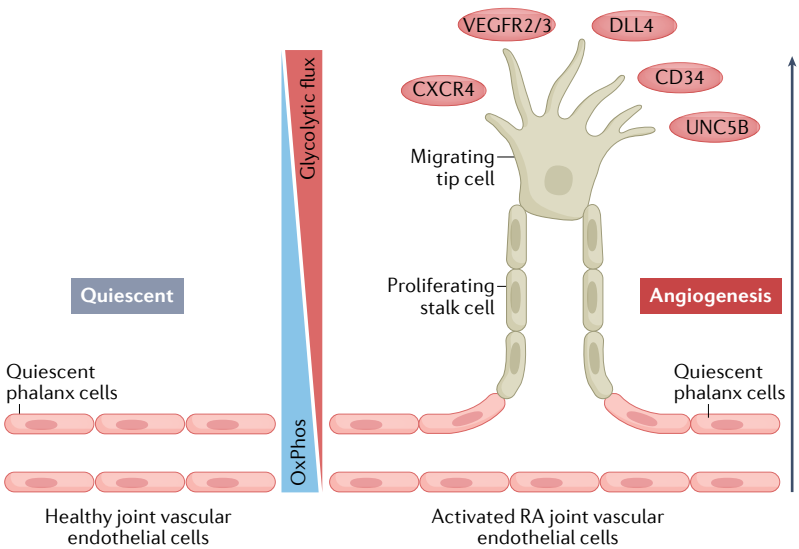


Fig. 2 | **Metabolic requirements of endothelial tip, stalk and phalanx cells.** Metabolic comparison of the three main microvascular endothelial cell subsets: the tip, stalk and phalanx cells. Their metabolic profiles are determined by their specific functions (migration, proliferation and quiescence, respectively). Angiogenesis requires a guiding tip cell, which is highly migratory and defined by a molecular signature of the Notch ligand Delta-like protein 4 (DLL4), the surface adhesion glycoprotein CD34, the axon guidance receptor UNC5B, neuropilin 1, CXCR4 and vascular endothelial growth factor (VEGF) receptors 2 and 3 (VEGFR2 and VEGFR3), which are expressed on rheumatoid arthritis (RA) synovial vasculature. Glycolysis is required for this guiding tip cell to direct new vessel formation. OxPhos, oxidative phosphorylation.

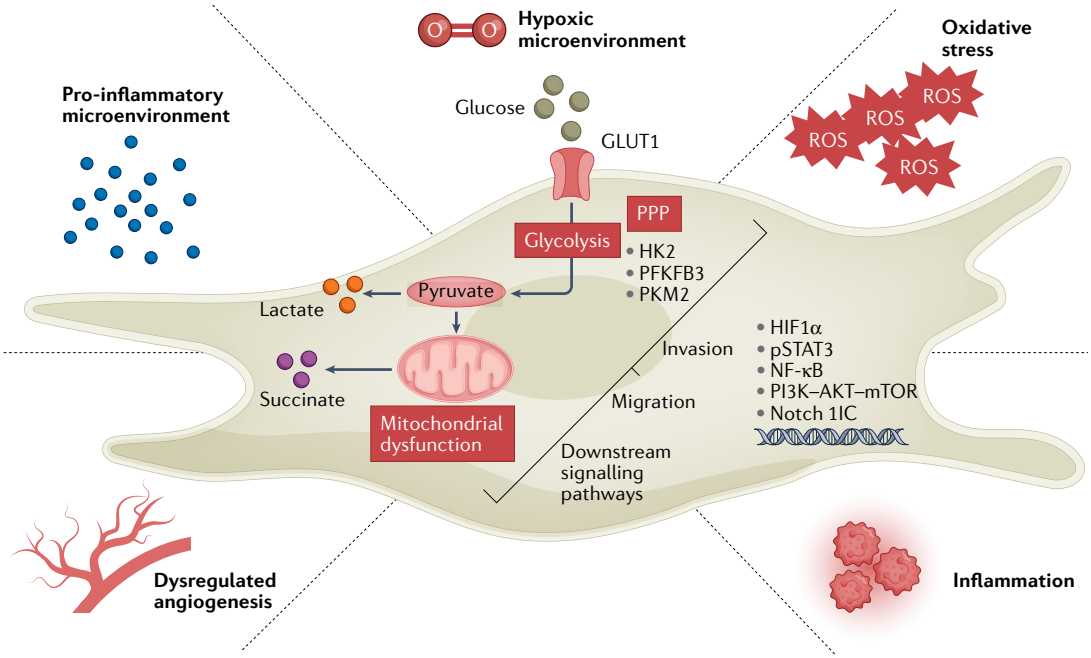


Fig. 3 | **Metabolic reprogramming of synovial fibroblasts in rheumatoid arthritis.** The inflammatory hypoxic microenvironment of the synovium in rheumatoid arthritis (RA), coupled with angiogenesis and oxidative stress, leads to glucose transporter 1 (GLUT1) upregulation, which enhances glucose uptake and glycolysis in RA synovial fibroblasts. Glycolytic enzymes such as hexokinase 2 (HK2), 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3 (PFKFB3) and pyruvate kinase M2 (PKM2) have key roles in promotion of the metabolic shift in favour of glycolysis. Production of metabolic intermediates such as lactate and succinate is also increased, along with mitochondrial dysfunction. Together, these processes result in an invasive, aggressive phenotype in RA synovial fibroblasts, and upregulation of downstream signalling factors such as hypoxia-inducible factor 1α (HIF1α), pSTAT3, NF-κB, PI3K–AKT–mTOR and Notch 1 intracellular domain (Notch 1IC). PPP, pentose phosphate pathway; ROS, reactive oxygen species.

6-phosphate dehydrogenase (a rate-limiting enzyme of the PPP) than wild-type cells, suggesting diversion of glucose-derived carbon to the PPP to sustain rapid proliferation of the tip–stalk cell phenotype. Upregulation of expression of glucose 6-phosphate isomerase (G6PI) also occurs in synovial endothelial cells, and G6PI silencing demonstrates that hypoxia-induced angiogenesis in RA is dependent on G6PI[71]. Furthermore, in a collagen-induced arthritis (CIA) model, succinate induces synovial neoangiogenesis via VEGF-dependent HIF1α pathways[72], and lactate can induce the secretion of angiogenic growth factors from RA synovial fibroblasts[23], which in turn can activate pro-angiogenic processes.

Endothelial cells express MHC class I and class II complexes on their surfaces, can present antigen and express the co-stimulatory and co-inhibitory molecules intercellular adhesion molecule 1, vascular cell adhesion protein 1, inducible co-stimulator-ligand and programmed death-ligand 1, and thus can activate T cell proliferation and differentiation[73,74]. Because endothelial cells rely heavily on glycolysis, they secrete considerable amounts of lactate, and higher concentrations of lactate are found in RA joints than in healthy tissues[23,75]. Sodium lactate selectively regulates $CD4^+$ T cell function, whereas lactic acid modulates $CD8^+$ T cells, which leads to entrapment of $CD4^+$ T cells within the inflamed synovial compartment[76]. Lactate uptake into $CD4^+$ T cells induces IL-17 production via PKM2–STAT3 interaction and fatty acid synthesis[51]. Endothelial cell-derived metabolites such as lactate, nitric oxide (NO), indoleamine 2,3-dioxygenase and sphingosine 1-phosphate have important roles in orchestrating T cell metabolic reprogramming[75].

Synovial fibroblasts and metabolism

Synovial fibroblasts are the resident mesenchymal cells of RA synovial tissue[77]. Synovial fibroblasts produce the extracellular matrix components that are essential for the structural framework of the synovial tissue and for cartilage integrity[77]. In RA, synovial fibroblasts are transformed into an aggressive pathogenic phenotype, with enhanced capacity to migrate to, adhere to and invade articular cartilage, and to secrete pro-inflammatory mediators, including receptor activator of NF-κB ligand, which induces bone resorption[77,78]. The destructive phenotype of RA synovial fibroblasts is associated with a shift in their metabolic profile (FIG. 3), with changes in glycolysis, the PPP, oxidative phosphorylation and amino acid metabolism relative to OA synovial fibroblasts[23,31,48,79]. RA synovial fibroblasts are reliant on both glucose and glutamine metabolism to support anabolic processes of lipids and nucleic acids[23,79–81], and in vitro studies have shown that conditions with deficiencies of glucose or glutamine inhibit RA synovial fibroblast proliferative and invasive functions[23,79–81]. Consistently, several studies have shown that the microenvironment of the inflamed joint, including low

pO_2 (REF.[16]), oxidative stress[82] and pro-inflammatory mediators, promotes a metabolic shift in RA synovial fibroblasts in favour of glycolysis, which is paralleled by upregulation of the expression of glycolytic enzymes including hexokinase 2, PFKFB3, PKM2 and GLUT1[23,47,65,79] (FIG. 3). Mitochondrial dysfunction and alteration of mitochondrial gene expression associated with apoptosis, redox balance and mitochondrial protein transport also occur in RA synovial fibroblasts following stimulation with TNF and TLR2 ligands[23]. Blockade of glycolytic enzymes, including PFKFB3 (REF.[23]) and hexokinase 2 (REF.[79]), reduces the capacity of RA synovial fibroblasts to migrate and invade, and limits secretion of pro-inflammatory mediators and activation of downstream transcriptional pathways involving HIF1α, pSTAT3, NF-κB and Notch 1IC[23]. In addition, the PI3K–AKT–mTOR intracellular signalling pathway, which is important for regulation of cell growth and proliferation, is implicated in mediation of the invasive functional capacity of RA synovial fibroblasts. Induction of the expression of the amino acid transporter SLC7A5 in RA synovial fibroblasts promotes secretion of matrix metalloproteinase 3 (MMP3) and MMP13 through NF-κB and mTOR–ribosomal protein S6 kinase β1 signalling activity[83], and the amino acid transporter LAT1 mediates IL-17-induced RA synovial fibroblast migration through mTOR signalling[84]. In addition, TNF signalling can co-opt the mTOR pathway to induce IFNγ responses in RA synovial fibroblasts through differential NF-κB and STAT1 signalling, an effect that is dependent on amino acid availability[85]. IL-1β-induced MMP3 and MMP13 expression is inhibited by silencing of the regulatory-associated protein of mTOR (Raptor, a component of mTORC1)[86], and the invasive capacity of RA synovial fibroblasts that involves regulation of cytoskeletal signalling pathways is suppressed by mTOR blockade[87]. Activation of complement C3 and C3a receptor induces synovial fibroblast activation via induction of metabolic pathways mediated by mTOR and HIF1α[88]. Metabolomic profiling has identified alteration of lipid metabolism in RA synovial fibroblasts compared with OA synovial fibroblasts[31,79,80]. Furthermore, the choline pathway, which can interact with lipids, is highly activated in RA synovial fibroblasts[89,90], and choline kinase-α inhibition suppresses migrative and invasive mechanisms in cultured RA synovial fibroblasts and ameliorates inflammation in the K/BxN mouse model of arthritis in vivo[89,90].

Within the joint environment, RA synovial fibroblasts interact with many different immune cells and have the potential through cell–cell interactions to further exacerbate the inflammatory response. Indeed, the expression of specific HLA-DR alleles on RA synovial fibroblasts provides evidence for a key role in their regulation of T cells[91]. Specifically, cell–cell interactions between T cells and synovial fibroblasts induce reciprocal changes in their metabolic profiles, with a shift towards aerobic glycolysis for both cell types[92], and concomitant increases in amounts of lactate, pro-inflammatory cytokines, adhesion molecules, VEGF and matrix-degrading enzymes[17,92]. Co-culture with RA synovial fibroblasts increases the percentages of $CD4^+$ T cells that produce TNF, IFNγ and IL-17A, and conditioned media from culture of $CD4^+$ T cells promote RA synovial fibroblast invasiveness, which is accompanied by an increase in glycolytic capacity[92]. Notably, the reciprocal regulation of RA synovial fibroblasts and T cells is inhibited by JAK–STAT inhibition[17,93], and by glycolytic inhibitors including 2-deoxyglucose and the AMP analogue 5-aminoimidazole-4-carboxamide ribonucleotide[92]. Crosstalk also occurs between RA synovial fibroblasts and synovial macrophages, such that conditioned medium from RA synovial fibroblasts induces both glycolysis and mitochondrial respiration in synovial macrophages, accompanied by an increase in their inflammatory responses[94]. Furthermore, metabolic intermediates such as succinate, which accumulates in macrophages, are known to activate RA synovial fibroblast invasiveness[23], and itaconate (an inhibitor of succinate with distinct roles that depend on physiological context) has both pro-inflammatory and anti-inflammatory effects on macrophages[32]. RA synovial fibroblasts also express the lactate transporter MCT4, knockdown of which induces RA synovial fibroblast apoptosis, and the use of MCT4-specific small interfering RNA in mouse models of CIA results in reduction in the severity of arthritis[19]. Results from studies using single-cell RNA sequencing and epigenetics analysis demonstrate that synovial fibroblast subsets exist within inflamed joints in RA, and these subsets have divergent functional roles and could differ in their metabolic requirements[95,96]. Anatomical location could also contribute to this diversity[96].

Metabolism and innate immunity

Myeloid cells (monocytes and macrophages) are present in large numbers in inflamed joints in RA and are innate effector cells that contribute to the initiation and perpetuation of inflammation. The association between metabolic changes and macrophage activation is well established, and alteration of amino acid metabolism was one of the first discriminatory features used for the definition of polarized macrophages in vitro. Macrophages with a pro-inflammatory phenotype are described as M1-like, and they metabolize arginine to NO via the activity of inducible NO synthase and citrulline. By contrast, homeostatic or M2-like macrophages metabolize arginine via arginase 1 activity[52,97]. An imbalance in macrophage subsets in favour of pro-inflammatory macrophages is thought to be a major cause of RA disease progression[98]. Moreover, activated M1-like macrophages have high requirements for glucose[99], and their metabolism switches from oxidative phosphorylation to glycolysis in response to inflammatory triggers such as Toll-like receptors[99]. In addition, the TCA cycle in inflammatory macrophages breaks down at two key steps (isocitrate dehydrogenase and succinate dehydrogenase), leading to accumulation of isocitrate and succinate[99–102]. The combination of enhanced glycolytic flux coupled with a disrupted TCA cycle is an activation hallmark of M1-like macrophages, and is associated with their inflammatory functional phenotype[103] (FIG. 4). By contrast, M2-like macrophages depend on oxidative phosphorylation, and have no TCA-cycle disruptions[104].

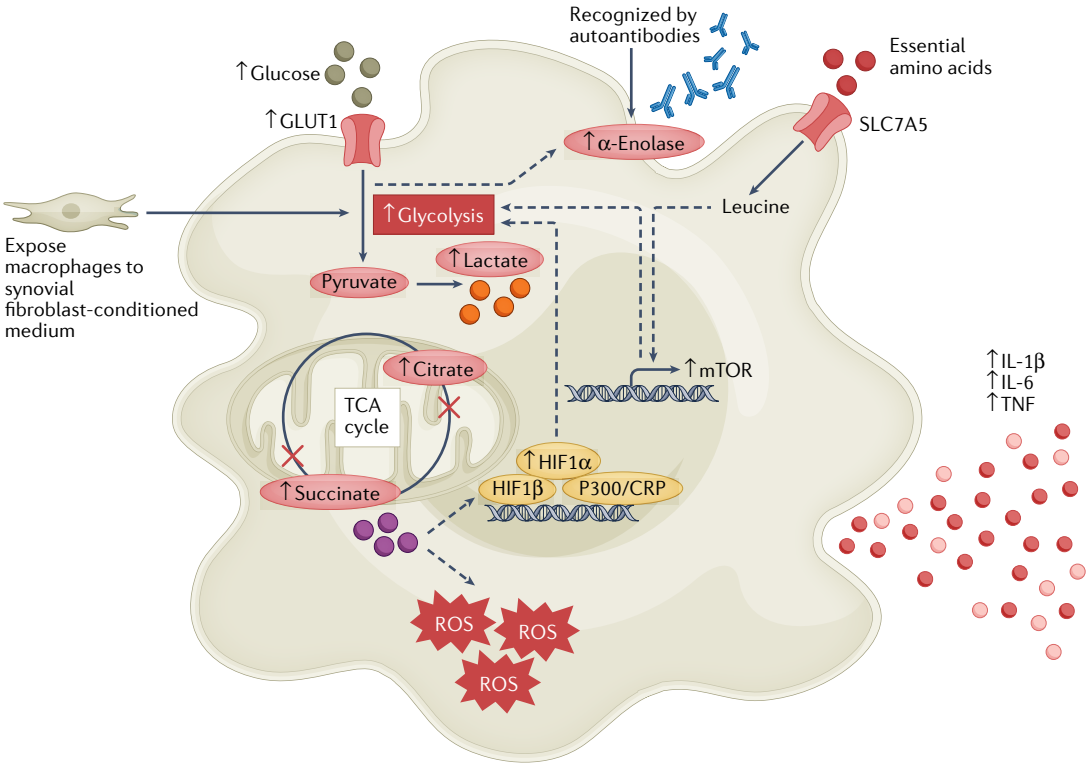


Fig. 4 | **Metabolic adaptions of macrophages in rheumatoid arthritis.** Inflammatory macrophages are characterized by enhanced glycolytic rates, with increased glucose uptake and expression of glucose transporter 1 (GLUT1), and a fragmented tricarboxylic acid (TCA) cycle. Metabolic intermediates such as lactate, α-enolase, citrate and succinate have distinct roles in perpetuating the inflammatory response. α-Enolase is recognized by autoantibodies, thereby promoting cytokine production. Accumulation of succinate results in production of reactive oxygen species (ROS) and stabilization of hypoxia-inducible factor 1α (HIF1α), which can in turn promote glycolysis, resulting in a positive feedback loop. Compared with healthy individuals, expression of the amino acid transporter SLC7A5 is elevated in macrophages in patients with rheumatoid arthritis, and stimulates leucine-mediated mTOR signalling. All these processes combine to enhance expression of pro-inflammatory mediators such as IL-1β, IL-6 and TNF to promote inflammatory functions of macrophages within the inflamed synovium.

Sustained pro-inflammatory activity in glycolytic macrophages is important in the context of RA. CD68 is a marker of synovial macrophages in RA[105]. CD68$^+$ synovial macrophages correlate with synovial glycolytic enzyme activity, mitochondrial dysfunction and oxidative stress in vivo[8,23], and are inversely associated with synovial pO_2 in vivo[8,41,104]. These effects are reversed in patients with RA who respond to treatment with TNF inhibitors (numbers of CD68$^+$ macrophages are reduced in TNF inhibitor responders), whereas a combination of TNF and hypoxic conditions increases macrophage survival[8,106]. Reflecting the hypermetabolic status of the RA myeloid compartment, concentrations of lactate, citrate and succinate are also elevated in the RA synovium[31]. Succinate accumulation in macrophages results in HIF1α stabilization, which promotes production of IL-1β[101]. Not surprisingly, mice lacking the succinate receptor 1 (GPR91) display reduced macrophage activation and cytokine production in a model of antigen-induced arthritis[55]. Itaconate, a metabolic inhibitor of succinate dehydrogenase, is capable of modulating succinate levels, is elevated in the RA joint and correlates with RA disease activity and response to treatment[32,102]. *IRG1*, which encodes an enzyme that catalyses the conversion of aconitate to itaconate, is one of the most robustly induced genes in activated macrophages, highlighting the importance of the citrate–itaconate axis[107–109]. However, the role of itaconate in RA pathogenesis is highly debated, and evidence exists of both immunoregulatory and inflammatory functions for this bactericidal metabolite[32,40,109]. Moreover, macrophages in RA have high expression of α-enolase, a glycolytic enzyme that is recognized by autoantibodies. This recognition promotes cytokine production. In addition, high concentrations of glucose promote IL-1β secretion in RA myeloid cells via NLRP3 (REFS[50,110]).

Metabolic plasticity in myeloid cells widens the possibility of targeting bioenergetics for therapeutic benefit in RA. Myeloid HIF1α deletion results in a reduction of infiltration of macrophages and of joint swelling in models of CIA as a result of a decrease in macrophage mobility and invasive capacity[111]. Amino acid transporters, such as SLC7A5, which mediate uptake of many essential amino acids, are also implicated in inflammation. SLC7A5-mediated amino acid influx is associated with RA disease pathogenesis, and elevation of expression of SLC7A5 in RA monocytes correlates positively with measurements of C-reactive protein and erythrocyte

sedimentation rate[112]. Moreover, blockade or downregulation of SLC7A5 in RA monocytes and macrophages reduces IL-1β production and glycolysis[112].

Analysis of circulating monocytes in patients with RA demonstrates the presence of metabolic abnormalities that are associated with inflammatory function[37]. Circulating monocytes are primed for hyperinflammation and hyper-metabolism concomitant with upregulation of glycolytic mechanisms, demonstrating a fundamental abnormality in the processing of glucose. Interestingly, this distinct phenotype is evident prior to clinical manifestations of disease in circulating monocytes from individuals at risk of developing RA[37]. In line with this result, monocyte-derived macrophages in coronary artery disease (CAD) can 'memorize' both the metabolic and inflammatory signatures of their precursor monocytes[113] via dimerization of PKM2, which subsequently functions as a protein kinase to phosphorylate and activate STAT3. Activation of STAT3 boosts secretion of pro-inflammatory cytokines such as IL-6 and IL-1β, which are encoded by genes with STAT3 binding sites in their promoters[114]. Evidence from studies of cancer indicates that dimeric PKM2 can use phosphoenolpyruvate as a phosphate donor in the phosphorylation of tyrosine residues in the activation of STAT3[115–117]. This metabolic dysfunction might be a signature of inflammation, as monocyte-derived macrophages in RA and CAD demonstrate similar metabolic disruption to healthy individuals[118]. In both RA and CAD, macrophages produce reactive oxygen species (ROS) through suppression of glycogen synthase kinase 3β (GSK3β), an inhibitor of glycogen synthase, which fuels mitochondrial activity[118]. However, differential glucose requirements of activated macrophages also define disease-specific signatures, indicating that myeloid metabolic abnormalities are not common to all autoimmune disorders[119]. Monocytes also seem to have immune memory mechanisms that act via epigenetic reprogramming, which is a potential therapeutic target in atherosclerosis[120]. The emerging discipline of 'metaboloepigenetics', which defines epigenetic–metabolic crosstalk, is interesting in this context. Notably many metabolites, such as acetyl-CoA, NAD, α-ketoglutarate and ATP, serve as cofactors for enzymes involved in chromatin remodelling[121]. Epigenetics can translate metabolic rewiring into alteration of gene expression and macrophage function[122,123]. In RA, many abnormal epigenetic modifications occur, and stromal cell epigenetic alteration is crucial for disease progression[124,125]. However, data relating to metaboloepigenetics in patients with RA are limited, and future studies could help to understand initiation, remission, relapse and chronic phases of the disease.

Cellular crosstalk between synovial tissue macrophages and synovial fibroblasts is recognized as an important interaction in regulation of joint inflammation. One subset of heparin-binding EGF-like growth factor (HBEGF)$^+$ macrophages found in the RA synovium can promote synovial fibroblast invasiveness[126]. Notably, synovial fibroblasts are also capable of shaping HBEGF$^+$ inflammatory macrophages, demonstrating the intricate crosstalk between these resident synovial cell types[126]. This crosstalk might also include metabolic interactions, and RA macrophages cultured in the presence of RA synovial fibroblast-conditioned medium demonstrate induction of glycolytic and mitochondrial respiration mechanisms, and uptake of glucose and glutamine[94]. In turn, succinate produced by activated macrophages can induce RA synovial fibroblast invasiveness[23].

Although macrophages can differentiate from circulating monocytes, it is now appreciated that synovial macrophages can also derive from embryonic precursors independent of haematopoiesis and that embryonically derived macrophages are capable of self-renewal in the steady state[127–130]. As macrophage activation involves crosstalk between metabolic reprogramming, epigenetic and transcriptional regulation[122,131], synovial tissue macrophages might possess some degree of metabolic flexibility. Infiltrating inflammatory macrophages might be short-lived, terminally differentiated cells that use glycolysis for rapid immune activation. By contrast, a short-term metabolic fix may not sustain self-renewing tissue-resident macrophages, and thus could have more sustainable metabolic profiles[122,132]. Macrophage metabolism might also vary according to the tissue-specific microenvironment[133], although evidence relating to RA synovial tissue macrophages is lacking.

DCs are involved at the interface of the innate and adaptive immune responses because of their roles in antigen presentation and cytokine production. DCs can be broadly classified into conventional myeloid and plasmacytoid cells[134] residing in the peripheral blood, with tissue-resident DCs in synovial tissue. The many subsets of DCs can be classified according to functional specialization, developmental origin or maturity status[135]. The inflammatory DC subset (which is probably monocyte-derived) is the main DC subset in synovial fluid in RA, and is associated with induction of the T helper 17 (T_H17) cell response[136]. By contrast, plasmacytoid DCs in the periphery are thought to have a more tolerogenic role in RA[137]. Moreover, CD141$^+$ DCs are enriched in inflamed synovial joints, and these synovial cells are activated and transcriptionally distinct from peripheral CD141$^+$ DCs[138].

In the resting state, DCs rely mainly on oxidative phosphorylation as their main source of energy to maintain their immature phenotype[139]. However, activated DCs utilize different metabolic pathways to supply sufficient energy to enable them to carry out diverse effector functions[140–142]. TLR stimulation enhances DC glycolysis, which supports chemotaxis, expression of pro-inflammatory mediators and antigen presentation[140–143]. Although initial TLR activation enhances glycolysis and maintains oxidative phosphorylation, long-term activation of DCs requires elevated glycolysis, which is associated with inhibition of oxidative phosphorylation[141,144]. The early boost in glycolysis is associated with increased expression of GLUT1 receptor and lactate production, whereas the later phases are associated with fatty acid synthesis and PPP metabolism[140–145]. Consistently, activated DCs do not become functionally mature in the presence of specific metabolic inhibitors[142]. In the SKG mouse model

of arthritis, treatment with 3-bromopyruvate, which directly targets GAPDH enzymatic activity (but can also covalently modify hexokinase 2), ameliorates disease through suppression of DC activation along with modulation of the balance between T_H17 cells and regulatory T (T_{reg}) cells[146].

The metabolic requirements of DCs in synovial joints in RA are not fully understood. The RA synovial microenvironment is capable of shaping DCs via alteration of their bioenergetic status during maturation, with elevation of the production of cytokines, co-stimulatory markers, chemokines and adhesion molecules, coupled with a metabolic shift towards enhanced glycolysis[147]. DCs can sense and respond to metabolites (such as succinate, butyrate and ATP) released by activated synovial cells in their environment[55,147–152], and succinate can promote DC migration via GPR91 activation[55,146–151]. GPR91-induced recruitment of DCs into lymph nodes contributes to the development of experimental antigen-induced arthritis through DC-facilitated T_H17 expansion[152]. These results highlight the key role of metabolism and metabolic intermediates in the induction and shaping of DC responses. Given the abundance of glucose transporters and glycolytic enzymes in the inflamed synovium in RA[23], DC glycolytic mechanisms could be an important therapeutic target.

The involvement of mast cells and innate lymphoid cells in synovial inflammation in RA is poorly understood, and their metabolism and contribution to the bioenergetics of the inflamed joint warrant further investigation[153,154]. Mast cells are present in the synovial tissue of patients with early RA and are associated with disease activity following treatment with DMARDs[153]. Mast cell metabolic requirements differ for activation and function, with initial activation being dependent on glycolysis, whereas cytokine production and degranulation require oxidative phosphorylation[155]. IL-9-expressing ILC2 innate lymphoid cells are implicated in the resolution of synovial inflammation via activation of T_{reg} cells[156]. As with mast cells, activation and function in ILC2 cells utilize different metabolic pathways, with cytokine production being dependent on mTOR activation and glycolysis[157].

Adaptive immune cells and metabolism

T cells have central roles in several autoimmune disorders, including RA, and understanding the involvement of metabolic pathways in T cell activation, expansion and polarization is important for the development of immunotherapeutics targeting metabolic processes[158]. Within minutes of T cell receptor (TCR)-mediated stimulation, pyruvate dehydrogenase kinase 1 is activated, and resting T cells rapidly upregulate aerobic glycolysis relative to oxidative phosphorylation[159]. This response is rapid because it is independent of transcription, and it can lead to sustained induction of glycolysis[57]. The emergence of naive T cells from this quiescent state is linked to metabolic adaptation to activation, and key decisions regarding cell fate and polarization occur during initial TCR engagement[160]. Although metabolic programming is not restricted to T cell polarization towards pathogenic IL-17-expressing T_H17 cells, these cells were the focus of several studies because of their evident involvement in multiple autoimmune disorders[161,162]. Notably, T_H cell polarization towards T_H17 or T_{reg} cells is associated with metabolic reprogramming[54,163]. Specifically, T_H17 polarization is linked to upregulation of glycolysis and HIF1α activity, whereas the same pathways suppress the generation of T_{reg} cells[54,163]. Furthermore, upon TCR engagement, upregulation of expression of basic leucine zipper transcription factor TF-like (BATF) and interferon-regulatory factor 4 (IRF4) leads to chromatin rearrangements that enable T_H17-related transcription factor RORγt expression[161]. BATF activity is linked to oxidative phosphorylation[164], the use of which at early stages following TCR engagement enhances BATF-dependent T_H17 polarization, whereas oligomycin-mediated inhibition of oxidative phosphorylation leads to reduction of T_H17 cell polarization and elevation of suppressive T_{reg} cell generation[164]. mTOR is central to the metabolic reprogramming that is required for T cells to exit a quiescent state and for their subsequent polarization[160]. mTORC1-dependent glycolysis is required for T cell IL-4-receptor expression, which enables T_H2 cell polarization. Inhibition of glycolysis or elimination of Raptor, leading to mTORC1 impairment, results in diminished T_H2 cell generation[160]. Importantly, the effect of metabolic pathway decision-making does not end with initial T cell polarization, as evidence suggests that metabolism is linked to T cell plasticity and the ability of T_H17 cells to transdifferentiate into T_H1-like cells[165]. T cells present in the synovial tissue of patients with RA are highly polyfunctional, and in particular, synovial ex-T_H17 T cells are resistant to T_{reg}-mediated suppression[166,167].

Intrinsic defects of T cell metabolism. T cell dysregulation in patients with RA could be a result of intrinsic defects affecting cell metabolism and leading to T cell plasticity, cytokine production, invasiveness and even pyroptotic cell death. Furthermore, defects in the rate-limiting glycolytic enzyme PFKFB3 occur in circulatory naive $CD4^+$ T cells from patients with RA, restricting the utilization of glucose for glycolysis so that it is instead channelled to the PPP[168]. This pathway leads to accumulation of NADPH and limits production of ROS, resulting in reductive stress for RA T cells[168]. The NADPH production is evidence of enhancement of lipid biosynthesis, which results in the accumulation of lipids in synovial tissue and activated memory T cells in RA[57]. The diversion away from glycolysis induces the expression of adapter protein TKS5, which promotes RA T cell invasive capacity, an effect that is reversed by inhibition of fatty acid synthesis[57]. Synovial-tissue T cells from patients with RA can produce multiple pro-inflammatory cytokines simultaneously, and this polyfunctionality is potentially linked to their metabolic profiles, as inhibition of oxidative phosphorylation results in reduction of cytokine production and polyfunctionality[166,169].

T cells from patients with RA are deficient in the nuclease MRE11A (part of the DNA repair machinery), resulting in cytosolic accumulation of damaged mitochondrial DNA and subsequent inflammasome-induced

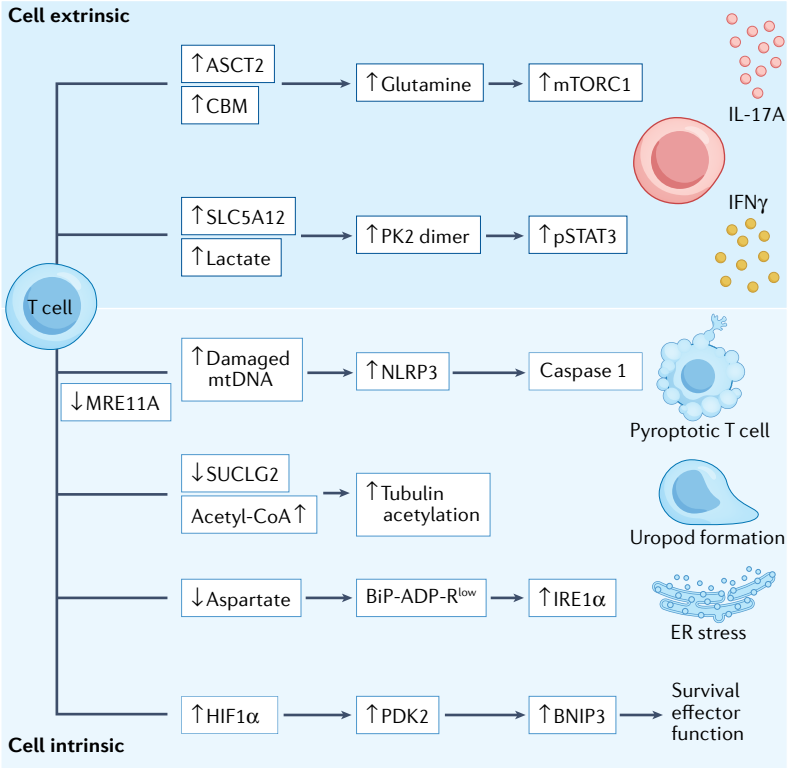


Fig. 5 | **Intrinsic and extrinsic mechanisms of metabolic adaptation and dysfunction of T cells in rheumatoid arthritis.** T cell-specific metabolic mechanisms contributing to synovial inflammation can be labelled as cell extrinsic or cell intrinsic. Cell-extrinsic mechanisms contribute to T cell metabolic dysfunction with increased uptake of lactate via lactate receptor SLC5A12 or glutamine via glutamine receptor alanine, serine, cysteine transporter 2 (ASCT2), resulting in production of IL-17A and IFNγ pro-inflammatory cytokines. Cell-intrinsic mechanisms include reduced function of the nuclease MRE11A, resulting in DNA damage, mitochondrial dysfunction and cytosolic accumulation of damaged mitochondrial DNA (mtDNA), leading to inflammasome activation and inflammatory cell death (pyroptosis). Another intrinsic mechanism of invasive, inflammatory T cells in rheumatoid arthritis is dependent on succinate-CoA ligase (SUCLG2), deficiency of which changes the direction of the tricarboxylic acid (TCA) cycle, resulting in accumulation of acetyl coenzyme A (acetyl-CoA), acetylation of the cytoskeleton and repositioning of the mitochondria. As a result, T cell polarization changes, favouring uropod formation and increased T cell migratory capacity. Notably, impaired mitochondrial fitness, resulting in reduced aspartate formation, can lead to endoplasmic reticulum (ER) stress and increased synthesis of proteins, including pro-inflammatory cytokines. Parallels to the adaptation of T cells to hypoxia between lupus nephritis and rheumatoid arthritis can be drawn, implicating hypoxia-inducible factor 1α (HIF1α) expression with T cell metabolic adaptation, fitness and effector function in the inflamed joint. BiP-ADP-R, ER chaperone BiP ADP ribosylation; BNIP3, BCL2/adenovirus E1B 19 kDa protein-interacting protein 3; CBM, CARD11–BCL10–MALT1 signalosome; IRE1α, inositol-requiring transmembrane kinase/endoribonuclease 1α; NLRP3, NOD-, LRR- and pyrin domain-containing protein 3; PDK2, pyruvate dehydrogenase kinase isoform 2; PK2, pyruvate kinase.

pyroptosis, and potentially contributing to the inflammatory environment of the RA joint[170]. This mechanism could be evidence of the premature aging of these T cells[171]. Metabolic abnormalities associated with the premature ageing of T cells might promote pathogenicity by enhancing memory T cell generation, cytokine production and tissue invasiveness[1,172]. T cell deficiency in the GDP-forming β subunit of succinate-CoA ligase (SUCLG2) results in the accumulation of acetyl-CoA and perinuclear repositioning of mitochondria as a result of tubulin acetylation[173]. Repositioning of the mitochondria changes the polarization of the cell and promotes uropod formation and potentially contributes to synovial tissue T cell invasiveness[173]. Interestingly, the impaired mitochondrial fitness of T cells from patients with RA can result in a reduction of aspartate synthesis, which is linked to increased endoplasmic reticulum (ER) size and protein synthesis, including enhanced secretion of TNF[174]. Therefore, ER-rich T cells are the dominant TNF producers in the inflamed joint[174].

Importantly, T cells do not act alone in RA pathogenesis and synovial inflammation. As described herein, stromal cell–T cell crosstalk can lead to reciprocal activation and metabolic changes. In addition to stromal cells, monocyte–macrophage–T cell interactions have been studied in RA. Macrophages can promote T cell recruitment to the joint and guide T cell polarization towards T_H17 cells[175–177]. In the absence of studies of the reciprocal coordination of metabolic pathways during macrophage–T cell interaction in RA, it is safe to assume that metabolic changes are prevalent, as both T cell motility and polarization are linked to metabolism[178].

Environmental contribution to T cell metabolism. In addition to cell-intrinsic metabolic defects, the unique metabolic environment of the inflamed joint can contribute to synovial T cell dysregulation. Lactate accumulates at sites of chronic inflammation, including the inflamed joint[51,179]. Upon activation, T cells upregulate expression of the lactate receptor SLC5A12 and facilitate lactate uptake. Lactate can enhance production of synovial inflammation-associated cytokines IL-17A and IFNγ by T cells, while reducing glucose uptake and increasing accumulation of acetyl-CoA[51]. Despite acetyl-CoA-induced mitochondrial relocation and uropod formation, lactate-mediated reduction of T cell glycolysis leads to possible T cell retention within the site of inflammation[42]. The synovial tissue of patients with RA has a distinct metabolite profile, and 20 metabolites (including glutamine) are potential biomarkers of RA[31]. TCR-dependent activation of mTORC1 requires rapid glutamine uptake. $CD4^+$ T cell glutamine uptake is dependent on the alanine, serine, cysteine transporter 2, and impairment of its activity results in reduction of T_H1 and T_H17 polarization, with no effect on T_{reg} cell generation[180]. A key feature of the inflamed synovial tissue environment is that oxygen availability (which is reduced in hypoxic tissue) correlates positively with immune-cell infiltration[16]. Although, overall, low oxygen availability inhibits T cell responses, specific T cell subsets thrive under hypoxic conditions[181]. Metabolic adaptation of T cells to hypoxic conditions is key for their survival at the site of inflammation[182]. In patients with lupus nephritis and in a murine model of the disease, kidney-infiltrating T cells express HIF1α, leading to regulation of proline metabolism and facilitating T cell survival and effector function[182]. Effector memory T cells are particularly resistant to the inhibitory effects of hypoxia, because they can maintain high glycolytic capacity through HIF1α induction[181]. Importantly, HIF1α can enhance T_H17 cell polarization via direct engagement of RORγt, and simultaneously diminish T_{reg} cell generation by promoting degradation of FOXP3 (REF.[183]).

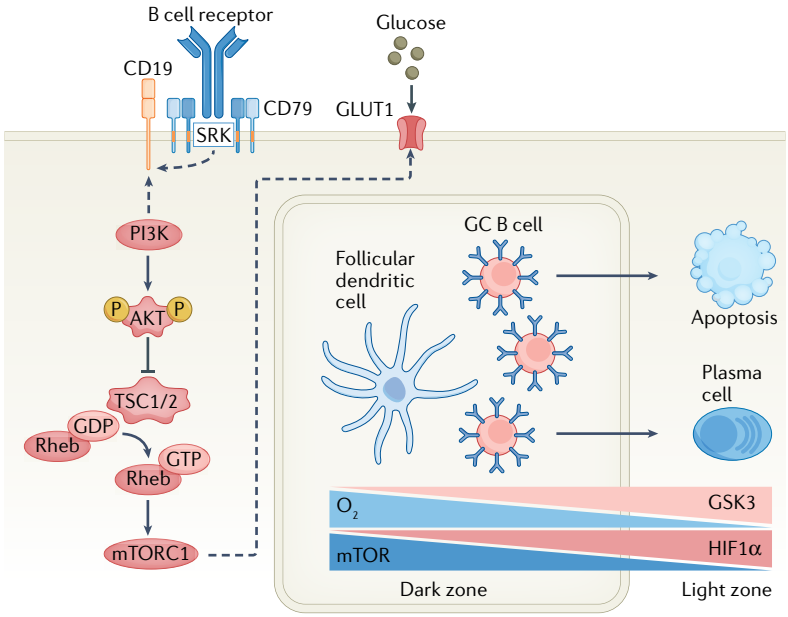


Fig. 6 | **Potential mechanisms of B cell metabolic programming in rheumatoid arthritis. a |** Despite the paucity of studies on B cell metabolism and synovial inflammation, we can extrapolate the importance of B cell metabolic programming from evidence of B cell germinal centre (GC) metabolic adaptation. Following B cell receptor engagement, PI3K is recruited, leading to the phosphorylation and activation of AKT, which phosphorylates the tuberous sclerosis complex (TSC1–TSC2) and inactivates its GTPase activity towards the GTP-binding protein Rheb, releasing Rheb to activate mTORC1. This pathway results in glucose transporter 1 (GLUT1) expression, glucose uptake and glycolysis. **b |** Following activation, GC B cells recirculate between the dark and light zones and must adapt to variable oxygen availability. In the dark zone, oxygen availability is high, B cells are highly proliferative and mTORC1 signalling is unimpeded. Under the hypoxic conditions of the light zone, B cells need to adapt to reduced oxygen and nutrient availability or risk apoptosis. Here, the mTORC1 pathway is restricted by high activity of hypoxia-inducible factor 1α (HIF1α), and glycogen synthase kinase 3 (GSK3) acts to induce a quiescent state and to shield B cells from metabolic collapse. Similar mechanisms could be implicated in the modulation of B cell functional and humoral immunity under the dynamic, hypoxic conditions of the inflamed joint.

Our current understanding of T cell metabolism in RA is summarized in FIG. 5, but further characterization of T cell metabolic dysregulation in chronic inflammation is needed for the identification of therapeutically targetable mechanisms that can reduce the immunopathology of RA.

B cell metabolism. Although inducible co-stimulator-ligand-expressing B cells can promote T cell polarization towards pathogenic T_H1 and T_H17 cells by altering the metabolic profiles of effector T cells in RA and systemic lupus erythematosus, the metabolic requirements of B cells and the interplay between B cell metabolism and function remain poorly understood[160]. Metabolism is key to several aspects of B cell biology, including activation, antibody production, survival and generation of long-lived plasma cells. Metabolic changes indirectly protect against the emergence of autoreactive B cells[184,185]. B cell receptor (BCR)-mediated B cell activation and the resulting increase in glycolysis and oxidative phosphorylation act as a 'time switch'. However, if the B cell fails to receive secondary signals in the form of TLR ligation or T cell help, then mitochondrial function becomes compromised, partly because of reduction of the activity of superoxide dismutase 2, mitochondrial, resulting in ROS accumulation and ROS-induced cell death[186]. Following activation, B cells enter the germinal centre, and if successful, undergo proliferation, class-switch recombination and somatic hypermutation, ultimately resulting in the generation of memory. These processes are enacted by the complex interplay between cell-intrinsic and environmentally orchestrated molecular programmes, and successful B cells have to adapt to the unique anatomical features of germinal centres[187], which are divided into two distinct anatomical structures, the light zone and the dark zone. B cell metabolic adaptation is an active process, and B cells migrate from the light zone to specialized sites of proliferation within the dark zone before re-entering the light zone[188], which is hypoxic. Light zone B cell proliferation and class-switch recombination is therefore limited[116]. Following BCR engagement, B cells utilize the mTOR pathway to increase GLUT1 expression and meet their demands for glucose uptake[189,190]. When glucose becomes scarce, glycogen synthase kinase 3 limits B cell proliferation and CD40-induced demand for glucose uptake, therefore protecting B cells from apoptosis resulting from limited nutrient availability[191]. In the hypoxic conditions of the light zone, induction of HIF limits mTOR pathway activity and expression of activation-induced cytosine deaminase, resulting in the restriction of light-zone B cell proliferation and class-switch recombination, whereas glycogen synthase kinase 3 protects B cells from ROS-induced apoptosis[191,192].

The limited available information relating to B cell metabolism in RA comes from studies of synovial T cells and the positive correlation between joint hypoxia and inflammation, and can be extrapolated to the potential role of B cell metabolism in the inflamed joint[16]. Notably, B cells derived from patients with RA are resistant to hypoxia-induced restriction of pro-inflammatory cytokine production or T cell co-stimulatory capacity[193]. Additionally, synovial tissue accumulates highly glycolytic B cells that express programmed cell death protein 1 (PD1), and these cells maintain higher mTOR activity and glucose uptake than PD1-negative B cells under hypoxic conditions that simulate the inflamed joint[193]. The synovial B cell metabolic state could also be influenced by CD40-mediated signals. CD40 and CD40L expression is higher in synovial tissue from patients with RA than in tissue from healthy individuals, and B cell expression of CD40L-responsive genes is similarly increased[194]. The formation of synovial ectopic lymphoid structures with germinal centre characteristics in RA also highlights that similar metabolic adaptations between germinal centres and synovial B cells might exist[195]. Because metabolism affects key aspects of B cell development and antibody production (FIG. 6), further research into B cell metabolism under the unique environmental conditions of the inflamed joint might lead to the identification of underlying pathogenic mechanisms, and ultimately novel therapeutic approaches.

Metabolism of other immune cells. Despite evidence of the involvement of synovial T cells in RA pathogenesis, the role of natural killer T (NKT) cells and

mucosal-associated invariant T cells is poorly understood. The limited available evidence suggests a reduction in numbers of NKT cells and alteration of the subpopulation distribution of mucosal-associated invariant T cells in the peripheral blood of patients with RA, compared with healthy individuals[196–198]. In murine models of RA, IL-17-expressing NKT cells contribute to synovial pathology, whereas IFNγ-producing NKT cells are mildly protective[199]. Interestingly, NKT cell metabolic adaptation during activation differs considerably from that of conventional T cells, because NKT cells metabolize glucose to the TCA cycle, and lactate-rich environments lead to a reduction of NKT cell proliferation and IL-17 production[200].

Targeting metabolism in RA

Considerable evidence indicates that altered metabolic pathways have important roles in defining cell phenotypes, and metabolic inhibition can lead to resolution of inflammation. However, metabolic requirements differ depending on the cell type, and indeed the cell subtype, in the synovium in RA and other diseases (FIG. 7). For instance, in RA, high lactate concentrations entrap $CD4^+$ T cells in the synovium by inhibiting their migratory capacity[51], whereas in the tumour microenvironment high lactate concentrations impair T cell function[201]. Similarly, although enolase and arginine induce monocyte–macrophage and DC activation in models of RA[50,140], they can also inhibit T cell function and promote T_{reg} activation[202–205]. Thus, understanding the competitive nutrient requirement for both immune and stromal cell subtypes in the inflamed RA joint is needed if we are to utilize metabolic pathways as potential therapeutic approaches[201]. However, many of our current treatment strategies can alter the metabolic profile of immune and stromal cells in RA. For instance, glucocorticoids regulate many genes associated with glycolysis, autophagy and mTOR pathways[206–208]. Conventional synthetic DMARDs for RA, including methotrexate, leflunomide and apremilast, are anti-metabolic through inhibition of purine and pyrimidine synthesis. This inhibition affects nucleotide formation, which is essential for cell proliferation, and thereby inhibits inflammation (BOX 1). Results from a metabolomic analysis of RA plasma demonstrated that glycolysis and amino acid metabolism predicted clinical response to methotrexate in early RA[209]. Biologic DMARDs, including TNF inhibitors, and targeted synthetic DMARDs, including the JAK inhibitor tofacitinib, alter metabolic pathways in favour of resolution of inflammation in vivo, ex vivo and in vitro[23,27,93,147]. Although the biologic DMARDs etanercept, canakinumab, secukinumab and tocilizumab have no effect on the increased glycolytic and inflammatory response observed in RA synovial fibroblasts following treatment with T_H cell-conditioned media, the JAK inhibitors tofacitinib and baricitinib and the glycolytic inhibitors 3-bromopyruvate and FX11 all suppress this RA synovial fibroblast response[17]. Furthermore, synovial

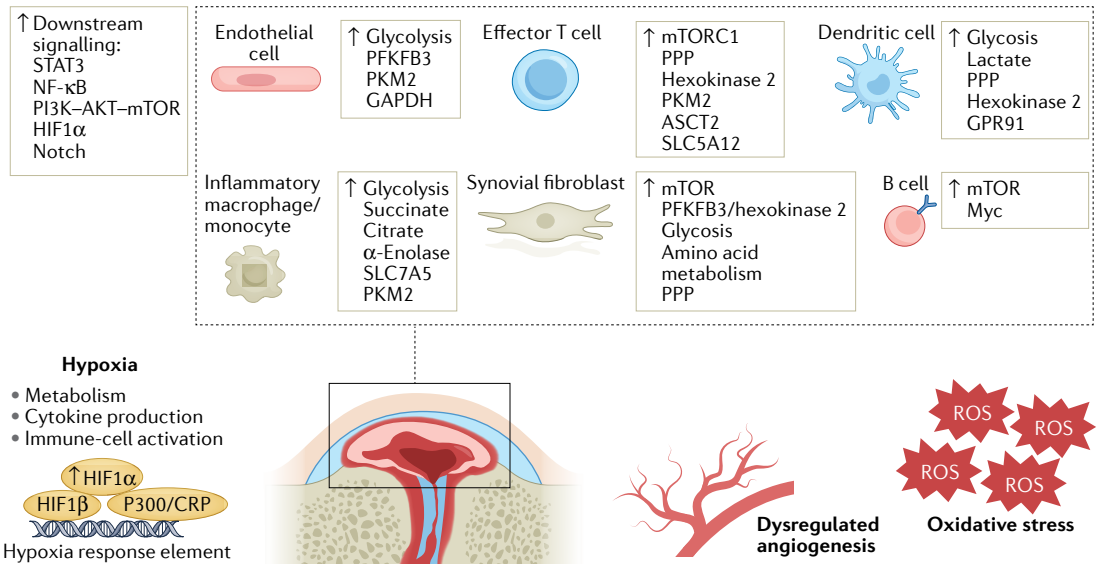


Fig. 7 | **Metabolic triggers for synovial cell subtypes.** Dysregulated angiogenesis, oxidative stress and the hypoxic microenvironment of the inflamed joint in rheumatoid arthritis force synovial cells to adapt and switch their cellular metabolism to maintain their pathogenic activated status. Specific metabolic triggers in some of the major cell types of the synovium are indicated, along with key signalling pathways. Enhanced glycolysis and glycolytic markers are observed in the endothelial cells and synovial fibroblasts, and the mTOR pathway is also involved in synovial fibroblast activation, along with amino acid metabolism and the pentose phosphate pathway (PPP). The tricarboxylic acid cycle intermediates citrate and succinate have a central role in the metabolic switch observed in inflammatory macrophages and activated monocytes, coupled with increased glycolytic flux. Dendritic cell metabolism can be directed by glycolysis, lactate production and the PPP. mTORC1, alanine, serine, cysteine transporter 2 (ASCT2), pyruvate kinase M2 (PKM2) and solute carrier family 5 member 12 (SLC5A12) are involved in the metabolic switch observed in effector T cells, whereas mTORC1 and Myc are important in B cells. GAPDH, glyceraldehyde 3-phosphate dehydrogenase; HIF1α, hypoxia inducible factor 1α; PFKFB3, 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3; ROS, reactive oxygen species; SLC7A5; solute carrier family 7 member 5.

Box 1 | **Metabolism-targeting therapeutic approaches in rheumatoid arthritis**

Current conventional synthetic DMARDs and biologic DMARDs used for treatment of patients with rheumatic diseases
- Reduce synovial fibroblast activation and secretion of pro-inflammatory mediators
- Reduced inflammation is associated with reduced metabolic activity in synovial biopsies before or after TNF inhibition
- Janus kinase–signal transducer and activator of transcription inhibition reduces metabolic and inflammatory pathway signalling in rheumatoid arthritis (RA) synovial fibroblasts, dendritic cells, monocytes and whole-tissue synovial explants
- Tocilizumab inhibits oxidative stress in RA leucocytes

Knockdown of the lactate transporter or use of lactate dehydrogenase inhibitors (such as FX11)
- Reduces RA synovial fibroblast invasiveness
- Inhibits T cell effector function
- Ameliorates joint inflammation in animal models of arthritis

Silencing of succinate receptor 1 or of tubulin acetyltransferase
- Inhibits macrophage activation
- Inhibits dendritic cell activation and T cell expansion
- Inhibits T cell effector function

Glutaminase 1 inhibition
- Inhibits T helper 17 (T_H17) cell expansion
- Reduces RA synovial fibroblast invasiveness
- Ameliorates severity of arthritis in animal models

Pyruvate kinase M2
- Pyruvate kinase M2 (PKM2) expression is increased in RA joints, and reduced in patients who respond to TNF inhibitors
- Reduction of PKM2 activity inhibits pro-inflammatory pathways in T cells and macrophages and inhibits chondrocyte function

PFKFB3
- Defects in 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3 (PFKFB3) in RA-naive $CD4^+$ T cells restrict the utilization of glucose for glycolysis so that it is diverted to the pentose phosphate pathway
- PFKFB3 inhibition reduces RA synovial fibroblast migration and invasive mechanisms
- PFKFB3 inhibition reduces endothelial cell tube formation and migration function

AMP-activated protein kinase inhibition (mTOR targeting)
- Inhibits RA synovial fibroblast invasiveness
- Inhibits T effector cell responses
- Reduces the ratio of T_H17 to regulatory T cells
- Inhibits osteoclast formation

3-bromopyruvate
- Reduces RA synovial fibroblast invasiveness
- Inhibits T effector cell function
- Ameliorates joint inflammation in animal models of arthritis

Inhibition of amino acid transporter SLC7A5
- Inhibits glycolysis and cytokine expression in human myeloid cells

fibroblasts from patients with resolving synovitis have greater mitochondrial respiratory capacity and intact mitochondrial morphology compared with synovial fibroblasts from patients with very early RA, suggesting that altering mitochondrial metabolites suppresses the RA synovial fibroblast invasive phenotype[210].

Many studies have examined targeting of specific metabolic pathways in models of RA[23,48,51,55,57,146] (BOX 1). The accumulation of lactate, succinate, citrate, itaconate and lipids, and their ability to regulate synovial fibroblast invasiveness[79], T cell differentiation and migration[54,57,112] and macrophage function[112,117], suggests the potential for targeting these metabolic intermediates, although caution is necessary as different cell types have different metabolic requirements. Furthermore, PKM2, which is upregulated in the RA joint[23], has roles in the function of T cells[113,211,212], macrophages[213] and chondrocytes[214]. Silencing glutaminase 1 causes inhibition of RA synovial fibroblast proliferation and reduction of the severity of experimental autoimmune arthritis[215]. Treatment with GSK2837808A, an inhibitor of lactate dehydrogenase A (LDHA), restores the decreased ATP:AMP ratio in activated OA synovial fibroblasts to baseline and can restore effector T cell function even in the presence of lactate[216,217]. Similarly, blockade of lactate transporter SLC5A12 results in amelioration of disease severity in a murine model of arthritis[51]. Use of the LDHA inhibitor FX11 or the GAPDH inhibitor 3-bromopyruvate can reduce lactate and pro-inflammatory cytokine production in T cell-stimulated synovial fibroblasts[17], as well as reducing synovitis in animal models of arthritis[48,146]. Notably, 3-bromopyruvate also has inhibitory effects on the glycolytic enzyme hexokinase 2 and on succinate dehydrogenase[146,218]. In studies investigating the roles of the TCA cycle and lipid metabolites, mice lacking the succinate receptor GPR91 displayed reduced macrophage activation and cytokine secretion, whereas inhibition of the choline kinase resulted in reduction of both migration and apoptosis resistance in RA synovial fibroblasts[55,89]. Pharmacological blockade of amino acid transporters such as SLC7A5 (expression of which correlates positively with RA disease parameters) results in inhibition of both glycolysis and cytokine expression in human myeloid cells[112].

Small molecular inhibitors of PFKFB3 reduce RA synovial fibroblast migration and invasion and the production of inflammatory mediators[23,219], but PFKFB3 is deficient in $CD4^+$ T cells in RA[168], again showing that metabolic enzymes can have differential effects that depend on the cell type. Silencing of hexokinase 1 or 2 inhibits the pro-inflammatory phenotypes of RA synovial fibroblasts and macrophages, in addition to leukocyte infiltration[220]. Fructose 1,6-bisphosphate, a glycolytic intermediate, has anti-inflammatory effects in animal models of arthritis, reducing joint swelling and secretion of pro-inflammatory cytokines, and enhancing IL-10 production[221]. The anti-diabetic drug metformin, which indirectly activates the energy sensor 5′ AMP-activated protein kinase, reduces inflammation in mouse models of arthritis[172] through inhibition of mTOR activity, alteration of autophagy and suppression of pro-inflammatory cytokine secretion[222]. Metformin also augments the effects of methotrexate in RA, suggesting that it has potential as an adjuvant therapy[223]. Finally, the combination of rapamycin and metformin reduces inflammation and the ratio of T_H17:T_{reg} cells and improves the metabolic profile of obese mice with CIA[224].

Targeting metabolism in preclinical RA

Defining preclinical autoimmunity (the concept that circulating autoantibodies are present long before disease onset) has become a major focus for rheumatology research. Autoantibodies such as

anti-citrullinated-protein antibodies (ACPA) and rheumatoid factor can precede clinical signs and symptoms of RA; thus, studying those individuals who are 'at risk' of developing RA could give valuable insights into disease evolution[225]. Notably, preclinical changes are often accompanied by early metabolic perturbations. Circulating monocytes in individuals at risk of developing RA are primed for hyper-metabolism accompanied by fundamental abnormalities in glucose processing, a phenotype shared by monocytes from patients with established RA[37]. Similarly, ACPA purified from patients with RA can induce an inflammatory phenotype in healthy monocyte-derived macrophages through release of IL-1β and ATP[226]. Preclinical metabolic changes are also observed in adaptive immune cells in RA. Results indicate that naive RA T_H cells are hypoglycolytic as a result of impaired expression of PFKFB3, a signature also observed in peripheral blood mononuclear cells from individuals at risk of developing RA[225,227].

Gut, lung or periodontal microbiome dysbiosis could herald a break of immune tolerance in RA via mechanisms of molecular mimicry or superantigen-dependent stimulation[228]. In addition to microbial dysbiosis, specific bacterial species could act as aetiological agents by harbouring peptidylarginine deiminase enzymes or by dysregulating these enzymes in the host, leading to upregulation of protein citrullination and neo-antigen formation that promotes HLA-DRB1-dependent formation of ACPA[229,230]. Intriguingly, in addition to the involvement of host–cell metabolism in RA, bacterial metabolism can lead to accumulation of metabolites with pro-inflammatory potential[231].

Conclusion

This Review of the roles of metabolic pathways in the various cell types found in RA synovial tissue provides a basis for the potential development of therapies that target metabolic pathways or specific metabolic intermediates, or for the identification of biomarkers. For instance, in a study of 'metabolic memory' in synovial fibroblasts from early RA and resolving synovitis, disease-associated bioenergetic changes were long lasting, with greater metabolic agility in response to inflammatory stimulation in synovial fibroblasts from patients with resolving arthritis[210]. Furthermore, alterations in metabolic pathways occur in monocytes and T cells early in disease[37,225,227], even in association with preclinical manifestations of disease. Differences are also seen between patients with RA who respond to biologic therapies and those who do not[17]. Thus, altered cellular bioenergetics might have a role in directing RA disease progression, but a better understanding of precisely how different synovial cell types orchestrate their metabolic demands at different stages of disease and outcomes is required. Furthermore, although there is clear evidence that altered metabolic pathways are involved in shaping the phenotype of both stromal and immune cells in RA, how these synovial cells compete for nutrient availability in the synovium is yet to be elucidated.

Further in-depth analysis of stromal and immune cell subtypes and their interactions at the site of inflammation will undoubtedly provide a deeper understanding of the metabolic perturbations and cell crosstalk, especially in whole synovial tissue, which might indicate novel therapeutic strategies. Currently, advanced technologies, including single-cell transcriptomics, cytometry by time of flight (CyTOF), cellular indexing of transcriptomes and epitopes by sequencing (CITE-seq), multiplexed immunohistochemistry such as co-detection by indexing (CODEX), and spatial transcriptomics, in tandem with advanced imaging technologies, are being applied to the synovium, as the target tissue of RA, to provide unparalleled insight into the cellular interactions and metabolic utilization at the site of inflammation.

Published online 19 April 2022

1. McInnes, I. B. & Schett, G. Pathogenetic insights from the treatment of rheumatoid arthritis. *Lancet* **389**, 2328–2337 (2017).
2. Tas, S. W., Maracle, C. X., Balogh, E. & Szekanecz, Z. Targeting of proangiogenic signalling pathways in chronic inflammation. *Nat. Rev. Rheumatol.* **12**, 111–122 (2016).
3. Ouyang, X. et al. Digoxin suppresses pyruvate kinase M2-promoted HIF-1alpha transactivation in steatohepatitis. *Cell Metab.* **27**, 339–350.e3 (2018).
4. Izquierdo, E. et al. Immature blood vessels in rheumatoid synovium are selectively depleted in response to anti-TNF therapy. *PLoS One* **4**, e8131 (2009).
5. Kennedy, A. et al. Angiogenesis and blood vessel stability in inflammatory arthritis. *Arthritis Rheum.* **62**, 711–721 (2010).
6. Fromm, S. et al. Enhanced angiogenic function in response to fibroblasts from psoriatic arthritis synovium compared to rheumatoid arthritis. *Arthritis Res. Ther.* **21**, 297 (2019).
7. Sivakumar, B. et al. Synovial hypoxia as a cause of tendon rupture in rheumatoid arthritis. *J. Hand Surg. Am.* **33**, 49–58 (2008).
8. Kennedy, A. et al. Tumor necrosis factor blocking therapy alters joint inflammation and hypoxia. *Arthritis Rheum.* **63**, 923–932 (2011).
9. Henderson, B., Bitensky, L. & Chayen, J. Glycolytic activity in human synovial lining cells in rheumatoid arthritis. *Ann. Rheum. Dis.* **38**, 63–67 (1979).
10. Treuhaft, P. S. & McCarty, D. J. Synovial fluid pH, lactate, oxygen and carbon dioxide partial pressure in various joint diseases. *Arthritis Rheum.* **14**, 475–484 (1971).
11. Naughton, D. P. et al. A comparative evaluation of the metabolic profiles of normal and inflammatory knee-joint synovial fluids by high resolution proton NMR spectroscopy. *FEBS Lett.* **332**, 221–225 (1993).
12. Troughton, P. R. et al. Synovial fluid interleukin-8 and neutrophil function in rheumatoid arthritis and seronegative polyarthritis. *Br. J. Rheumatol.* **35**, 1244–1251 (1996).
13. Lund-Olesen, K. Oxygen tension in synovial fluids. *Arthritis Rheum.* **13**, 769–776 (1970).
14. Etherington, P. J., Winlove, P., Taylor, P., Paleolog, E. & Miotla, J. M. VEGF release is associated with reduced oxygen tensions in experimental inflammatory arthritis. *Clin. Exp. Rheumatol.* **20**, 799–805 (2002).
15. Peters, C. L. et al. The transcription factors hypoxia-inducible factor 1α and Ets-1 colocalize in the hypoxic synovium of inflamed joints in adjuvant-induced arthritis. *Arthritis Rheum.* **50**, 291–296 (2004).
16. Ng, C. T. et al. Synovial tissue hypoxia and inflammation in vivo. *Ann. Rheum. Dis.* **69**, 1389–1395 (2010).
17. Kvacskay, P. et al. Increase of aerobic glycolysis mediated by activated T helper cells drives synovial fibroblasts towards an inflammatory phenotype: new targets for therapy? *Arthritis Res. Ther.* **23**, 56 (2021).
18. Ciurtin, C. et al. Correlation between different components of synovial fluid and pathogenesis of rheumatic diseases. *Rom. J. Intern. Med.* **44**, 171–181 (2006).
19. Fujii, W. et al. Monocarboxylate transporter 4, associated with the acidification of synovial fluid, is a novel therapeutic target for inflammatory arthritis. *Arthritis Rheumatol.* **67**, 2888–2896 (2015).
20. Maxwell, P. H. et al. The tumour suppressor protein VHL targets hypoxia-inducible factors for oxygen-dependent proteolysis. *Nature* **399**, 271–275 (1999).
21. Giatromanolaki, A. et al. Upregulated hypoxia inducible factor-1α and -2α pathway in rheumatoid arthritis and osteoarthritis. *Arthritis Res. Ther.* **5**, R193–R201 (2003).
22. Kaelin, W. & Ratcliffe, P. Oxygen sensing by metazoans: the central role of the HIF hydroxylase pathway. *Mol. Cell* **30**, 393–402 (2008).
23. Biniecka, M. et al. Dysregulated bioenergetics: a key regulator of joint inflammation. *Ann. Rheum. Dis.* **75**, 2192–2200 (2016).
24. Guo, X. & Chen, G. Hypoxia-inducible factor is critical for pathogenesis and regulation of immune cell functions in rheumatoid arthritis. *Front. Immunol.* **11**, 1668 (2020).
25. Oliver, K. M. et al. Hypoxia activates NF-κB-dependent gene expression through the canonical signaling pathway. *Antioxid. Redox Signal.* **11**, 2057–2064 (2009).
26. Li, C. et al. PI3 kinase/Akt/HIF-1α pathway is associated with hypoxia-induced epithelial-mesenchymal transition in fibroblast-like synoviocytes of rheumatoid arthritis. *Mol. Cell. Biochem.* **372**, 221–231 (2013).
27. Gao, W. et al. Hypoxia and STAT3 signalling interactions regulate pro-inflammatory pathways in rheumatoid arthritis. *Ann. Rheum. Dis.* **74**, 1275–1283 (2015).
28. Fearon, U., Canavan, M., Biniecka, M. & Veale, D. J. Hypoxia, mitochondrial dysfunction and synovial invasiveness in rheumatoid arthritis. *Nat. Rev. Rheumatol.* **12**, 385–397 (2016).

29. Du, H. et al. Tanshinone IIA suppresses proliferation and inflammatory cytokine production of synovial fibroblasts from rheumatoid arthritis patients induced by TNF-α and attenuates the inflammatory response in AIA mice. *Front. Pharmacol.* **11**, 568 (2020).
30. Qiu, J. et al. Metabolic control of autoimmunity and tissue inflammation in rheumatoid arthritis. *Front. Immunol.* **12**, 652771 (2021).
31. Kim, S. et al. Global metabolite profiling of synovial fluid for the specific diagnosis of rheumatoid arthritis from other inflammatory arthritis. *PLoS One* **9**, e97501 (2014).
32. Michopoulos, F. et al. Targeted metabolic profiling of the Tg197 mouse model reveals itaconic acid as a marker of rheumatoid arthritis. *J. Proteome Res.* **15**, 4579–4590 (2016).
33. Hanlon, M. M., Canavan, M., Barker, B. E. & Fearon, U. Metabolites as drivers and targets in rheumatoid arthritis. *Clin. Exp. Immunol.* https://doi.org/10.1093/cei/uxab021 (2021).
34. Liang, Y. et al. IL-33 activates mTORC1 and modulates glycolytic metabolism in CD8⁺ T cells. *Immunology* **165**, 61–73 (2022).
35. Rodriguez, A. E. et al. Serine metabolism supports macrophage IL-1beta production. *Cell Metab.* **29**, 1003–1011.e4 (2019).
36. Vigne, S. et al. IL-27-induced type 1 regulatory T-cells produce oxysterols that Constrain IL-10 production. *Front. Immunol.* **8**, 1184 (2017).
37. McGarry, T. et al. Rheumatoid arthritis CD14⁺ monocytes display metabolic and inflammatory dysfunction, a phenotype that precedes clinical manifestation of disease. *Clin. Transl. Immunol.* **10**, e1237 (2021).
38. Weyand, C. M. & Goronzy, J. J. Immunometabolism in the development of rheumatoid arthritis. *Immunol. Rev.* **294**, 177–187 (2020).
39. Weyand, C. M. & Goronzy, J. J. Immunometabolism in early and late stages of rheumatoid arthritis. *Nat. Rev. Rheumatol.* **13**, 1–11 (2017).
40. Pucino, V. et al. Metabolic checkpoints in rheumatoid arthritis. *Front. Physiol.* **11**, 347 (2020).
41. Buchakjian, M. & Kornbluth, S. The engine driving the ship: metabolic steering of cell proliferation and death. *Nat. Rev. Mol. Cell Biol.* **11**, 715–727 (2010).
42. Röhrig, F. & Schulze, A. The multifaceted roles of fatty acid synthesis in cancer. *Nat. Rev. Cancer* **16**, 732–749 (2016).
43. Harty, L. C. et al. Mitochondrial mutagenesis correlates with the local inflammatory environment in arthritis. *Ann. Rheum. Dis.* **71**, 582–588 (2012).
44. Gallagher, L. et al. Insulin-resistant pathways are associated with disease activity in rheumatoid arthritis and are subject to disease modification through metabolic reprogramming: a potential novel therapeutic approach. *Arthritis Rheumatol.* **72**, 896–902 (2020).
45. Lindy, S., Uitto, J., Turto, H., Rokkanen, P. & Vainio, K. Lactate dehydrogenase in the synovial tissue in rheumatoid arthritis: total activity and isoenzyme composition. *Clin. Chim. Acta* **31**, 19–23 (1971).
46. Pejovic, M., Stankovic, A. & Mitrovic, D. R. Lactate dehydrogenase activity and its isoenzymes in serum and synovial fluid of patients with rheumatoid arthritis and osteoarthritis. *J. Rheumatol.* **19**, 529–533 (1992).
47. McGarry, T. et al. Resolution of TLR2-induced inflammation through manipulation of metabolic pathways in rheumatoid arthritis. *Sci. Rep.* **7**, 43165 (2017).
48. Bustamante, M. et al. Hexokinase 2 as a novel selective metabolic target for rheumatoid arthritis. *Ann. Rheum. Dis.* **77**, 1636–1643 (2018).
49. Chang, X. & Wei, C. Glycolysis and rheumatoid arthritis. *Int. J. Rheum. Dis.* **14**, 217–222 (2011).
50. Bae, S. et al. α-Enolase expressed on the surfaces of monocytes and macrophages induces robust synovial inflammation in rheumatoid arthritis. *J. Immunol.* **189**, 365–372 (2012).
51. Pucino, V. et al. Lactate buildup at the site of chronic inflammation promotes disease by inducing CD4⁺ T cell metabolic rewiring. *Cell Metab.* **30**, 1055–1074.e8 (2019).
52. Modolell, M., Corraliza, I. M., Link, F., Soler, G. & Eichmann, K. Reciprocal regulation of the nitric oxide synthase/arginase balance in mouse bone marrow-derived macrophages by TH1 and TH2 cytokines. *Eur. J. Immunol.* **25**, 1101–1104 (1995).
53. Schaller, M., Burton, D. R. & Ditzel, H. J. Autoantibodies to GPI in rheumatoid arthritis: linkage between an animal model and human disease. *Nat. Immunol.* **2**, 746–753 (2001).
54. Shi, L. Z. et al. HIF1alpha-dependent glycolytic pathway orchestrates a metabolic checkpoint for the differentiation of TH17 and Treg cells. *J. Exp. Med.* **208**, 1367–1376 (2011).
55. Littlewood-Evans, A. et al. GPR91 senses extracellular succinate released from inflammatory macrophages and exacerbates rheumatoid arthritis. *J. Exp. Med.* **213**, 1655–1662 (2016).
56. Jung, S. et al. Ethyl pyruvate ameliorates inflammatory arthritis in mice. *Int. Immunopharmacol.* **52**, 333–341 (2017).
57. Shen, Y. et al. Metabolic control of the scaffold protein TKS5 in tissue-invasive, proinflammatory T cells. *Nat. Immunol.* **18**, 1025–1034 (2017).
58. Fearon, U., Veale, D. J. & Szekanecz, Z. in *Firestein and Kelley's Textbook of Rheumatology* 11th edn. (ed. Koretzky, G.) 445–454 (Elsevier, 2020).
59. Eelen, G. et al. Endothelial cell metabolism. *Physiol. Rev.* **98**, 3–58 (2018).
60. Cruys, B. et al. Glycolytic regulation of cell rearrangement in angiogenesis. *Nat. Commun.* **7**, 12240 (2016).
61. Gerhardt, H. et al. VEGF guides angiogenic sprouting utilizing endothelial tip cell filopodia. *J. Cell Biol.* **161**, 1163–1177 (2003).
62. del Toro, R. et al. Identification and functional analysis of endothelial tip cell-enriched genes. *Blood* **116**, 4025–4033 (2010).
63. Strasser, G. A., Kaminker, J. S. & Tessier-Lavigne, M. Microarray analysis of retinal endothelial tip cells identifies CXCR4 as a mediator of tip cell morphology and branching. *Blood* **115**, 5102–5110 (2010).
64. Goveia, J., Stapor, P. & Carmeliet, P. Principles of targeting endothelial cell metabolism to treat angiogenesis and endothelial cell dysfunction in disease. *EMBO Mol. Med.* **6**, 1105–1120 (2014).
65. Hanlon, M. M. et al. STAT3 mediates the differential effects of oncostatin M and TNFα on RA synovial fibroblast and endothelial cell function. *Front. Immunol.* **10**, 2056 (2019).
66. Akhavani, M. A. et al. Hypoxia upregulates angiogenesis and synovial cell migration in rheumatoid arthritis. *Arthritis Res. Ther.* **11**, R64 (2009).
67. Gao, W. et al. Notch-1 mediates hypoxia-induced angiogenesis in rheumatoid arthritis. *Arthritis Rheum.* **64**, 2104–2113 (2012).
68. Mor, I., Cheung, E. C. & Vousden, K. H. Control of glycolysis through regulation of PFK1: old friends and recent additions. *Cold Spring Harb. Symp. Quant. Biol.* **76**, 211–216 (2011).
69. Schoors, S. et al. Partial and transient reduction of glycolysis by PFKFB3 blockade reduces pathological angiogenesis. *Cell Metab.* **19**, 37–48 (2014).
70. Wade, S. M. et al. Dysregulated miR-125a promotes angiogenesis through enhanced glycolysis. *EBioMedicine* **47**, 402–413 (2019).
71. Lu, Y. et al. Glucose-6-phosphate isomerase (G6PI) mediates hypoxia-induced angiogenesis in rheumatoid arthritis. *Sci. Rep.* **7**, 40274 (2017).
72. Li, Y. et al. Succinate induces synovial angiogenesis in rheumatoid arthritis through metabolic remodeling and HIF-1α/VEGF axis. *Free Radic. Biol. Med.* **126**, 1–14 (2018).
73. Hirosue, S. et al. Steady-state antigen scavenging, cross-presentation, and CD8⁺ T cell priming: a new role for lymphatic endothelial cells. *J. Immunol.* **192**, 5002–5011 (2014).
74. Dimou, P. et al. The human glomerular endothelial cells are potent pro-inflammatory contributors in an in vitro model of lupus nephritis. *Sci. Rep.* **9**, 8348 (2019).
75. Certo, M. et al. Endothelial cell and T-cell crosstalk: targeting metabolism as a therapeutic approach in chronic inflammation. *Br. J. Pharmacol.* **178**, 2041–2059 (2021).
76. Haas, R. et al. Lactate regulates metabolic and proinflammatory circuits in control of T cell migration and effector functions. *PLoS Biol.* **13**, 1002202 (2015).
77. Ospelt, C. Synovial fibroblasts in 2017. *RMD Open* **3**, e000471 (2017).
78. Lefevre, S. et al. Synovial fibroblasts spread rheumatoid arthritis to unaffected joints. *Nat. Med.* **15**, 1414–1420 (2009).
79. Bustamante, M. F., Garcia-Carbonell, R., Whisenant, K. D. & Guma, M. Fibroblast-like synoviocyte metabolism in the pathogenesis of rheumatoid arthritis. *Arthritis Res. Ther.* **19**, 110 (2017).
80. Ahn, J. K. et al. GC/TOF-MS-based metabolomic profiling in cultured fibroblast-like synoviocytes from rheumatoid arthritis. *Jt Bone Spine* **83**, 707–713 (2016).
81. Garcia-Carbonell, R. et al. Critical role of glucose metabolism in rheumatoid arthritis fibroblast-like synoviocytes. *Arthritis Rheumatol.* **68**, 1614–1626 (2016).
82. Balogh, E. et al. Oxidative stress impairs energy metabolism in primary cells and synovial tissue of patients with rheumatoid arthritis. *Arthritis Res. Ther.* **20**, 95 (2018).
83. Xu, J. et al. Intervening upregulated SLC7A5 could mitigate inflammatory mediator by mTOR-P70S6K signal in rheumatoid arthritis synoviocytes. *Arthritis Res. Ther.* **22**, 200 (2020).
84. Weng, J., Tu, M., Yang, B. & Wang, Z. Relationship between LAT1 expression and resistance to chemotherapy in pancreatic ductal adenocarcinoma. *Cancer Chemother. Pharmacol.* **82**, 367 (2018).
85. Karonitsch, T. et al. mTOR senses environmental cues to shape the fibroblast-like synoviocyte response to inflammation. *Cell Rep.* **23**, 2157–2167 (2018).
86. Ito, M. et al. Selective interference of mTORC1/RAPTOR protects against human disc cellular apoptosis, senescence, and extracellular matrix catabolism with Akt and autophagy induction. *Osteoarthritis Cartilage* **25**, 2134–2146 (2017).
87. Laragione, T. & Gulko, P. S. mTOR regulates the invasive properties of synovial fibroblasts in rheumatoid arthritis. *Mol. Med.* **16**, 352–358 (2010).
88. Friscic, J. et al. The complement system drives local inflammatory tissue priming by metabolic reprogramming of synovial fibroblasts. *Immunity* **54**, 1002–1021.e10 (2021).
89. Guma, M. et al. Choline kinase inhibition in rheumatoid arthritis. *Ann. Rheum. Dis.* **74**, 1399–1407 (2015).
90. Seki, M. et al. Functional analysis of choline transporters in rheumatoid arthritis synovial fibroblasts. *Mod. Rheumatol.* **27**, 995–1003 (2017).
91. Held, G. et al. Differential presentation of tumor antigen-derived epitopes by MHC-class I and antigen-positive tumor cells. *Int. J. Cancer* **123**, 1841–1847 (2008).
92. Petrasca, A. et al. Targeting bioenergetics prevents CD4 T cell-mediated activation of synovial fibroblasts in rheumatoid arthritis. *Rheumatology* **59**, 2816–2828 (2020).
93. McGarry, T. et al. JAK/STAT blockade alters synovial bioenergetics, mitochondrial function, and proinflammatory mediators in rheumatoid arthritis. *Arthritis Rheumatol.* **70**, 1959–1970 (2018).
94. Saeki, N. & Imai, Y. Reprogramming of synovial macrophage metabolism by synovial fibroblasts under inflammatory conditions. *Cell Commun. Signal.* **18**, 188 (2020).
95. Croft, A. P. et al. Distinct fibroblast subsets drive inflammation and damage in arthritis. *Nature* **570**, 246–251 (2019).
96. Frank-Bertoncelj, M. et al. Epigenetically-driven anatomical diversity of synovial fibroblasts guides joint-specific fibroblast functions. *Nat. Commun.* **8**, 14852 (2017).
97. Murray, P. J. et al. Macrophage activation and polarization: nomenclature and experimental guidelines. *Immunity* **41**, 14–20 (2014).
98. Kurowska-Stolarska, M. & Alivernini, S. Synovial tissue macrophages: friend or foe? *RMD Open* **3**, e000527 (2017).
99. O'Neill, L. A. J. A broken krebs cycle in macrophages. *Immunity* **42**, 393–394 (2015).
100. Rodríguez-Prados, J.-C. et al. Substrate fate in activated macrophages: a comparison between innate, classic, and alternative activation. *J. Immunol.* **185**, 605–614 (2010).
101. Tannahill, G. M. et al. Succinate is an inflammatory signal that induces IL-1β through HIF-1α. *Nature* **496**, 238–242 (2013).
102. Lampropoulou, V. et al. Itaconate links inhibition of succinate dehydrogenase with macrophage metabolic remodeling and regulation of inflammation. *Cell Metab.* **24**, 158–166 (2016).
103. Freemerman, A. J. et al. Metabolic reprogramming of macrophages: glucose transporter 1 (GLUT1)-mediated glucose metabolism drives a proinflammatory phenotype. *J. Biol. Chem.* **289**, 7884–7896 (2014).
104. Van den Bossche, J., Baardman, J. & de Winther, M. P. J. Metabolic characterization of polarized M1 and M2 bone marrow-derived macrophages using real-time extracellular flux analysis. *J. Vis. Exp.* **105**, 53424 (2015).
105. Bresnihan, B. et al. Synovial tissue sublining CD68 expression is a biomarker of therapeutic response in rheumatoid arthritis clinical trials: consistency across centers. *J. Rheumatol.* **36**, 1800–1802 (2009).

106. Hamilton, J. A. et al. Hypoxia enhances the proliferative response of macrophages to CSF-1 and their pro-survival response to TNF. *PLoS One* **7**, e45853 (2012).
107. Jha, A. K. et al. Network integration of parallel metabolic and transcriptional data reveals metabolic modules that regulate macrophage polarization. *Immunity* **42**, 419–430 (2015).
108. Michelucci, A. et al. Immune-responsive gene 1 protein links metabolism to immunity by catalyzing itaconic acid production. *Proc. Natl Acad. Sci. USA* **110**, 7820–7825 (2013).
109. Papathanassiu, A. E. et al. BCAT1 controls metabolic reprogramming in activated human macrophages and is associated with inflammatory diseases. *Nat. Commun.* **8**, 16040 (2017).
110. Ruscitti, P. et al. Monocytes from patients with rheumatoid arthritis and type 2 diabetes mellitus display an increased production of interleukin (IL)-1β via the nucleotide-binding domain and leucine-rich repeat containing family pyrin 3(NLRP3)-inflammasome activation: a possible implication for therapeutic decision in these patients. *Clin. Exp. Immunol.* **182**, 35–44 (2015).
111. Cramer, T. et al. HIF-1α is essential for myeloid cell-mediated inflammation. *Cell* **112**, 645–657 (2003).
112. Yoon, B. R., Oh, Y. J., Kang, S. W. & Lee, L. E. Role of SLC7A5 in metabolic reprogramming of human monocyte/macrophage immune responses. *Front. Immunol.* **9**, 53 (2018).
113. Shirai, T. et al. The glycolytic enzyme PKM2 bridges metabolic and inflammatory dysfunction in coronary artery disease. *J. Exp. Med.* **213**, 337–354 (2016).
114. Samavati, L. et al. STAT3 tyrosine phosphorylation is critical for interleukin 1 beta and interleukin-6 production in response to lipopolysaccharide and live bacteria. *Mol. Immunol.* **46**, 1867–1877 (2009).
115. Yang, P., Li, Z., Fu, R., Wu, H. & Li, Z. Pyruvate kinase M2 facilitates colon cancer cell migration via the modulation of STAT3 signalling. *Cell Signal.* **26**, 1853–1862 (2014).
116. Yang, P. et al. Pyruvate kinase M2 accelerates pro-inflammatory cytokine secretion and cell proliferation induced by lipopolysaccharide in colorectal cancer. *Cell Signal.* **27**, 1525–1532 (2015).
117. Gao, X., Wang, H., Yang, J. J., Liu, X. & Liu, Z. R. Pyruvate kinase M2 regulates gene transcription by acting as a protein kinase. *Mol. Cell* **45**, 598–609 (2012).
118. Zeisbrich, M. et al. Hypermetabolic macrophages in rheumatoid arthritis and coronary artery disease due to glycogen synthase kinase 3b inactivation. *Ann. Rheum. Dis.* **77**, 1053–1062 (2018).
119. Watanabe, R. et al. Glucose metabolism controls disease-specific signatures of macrophage effector functions. *JCI Insight* **3**, e123047 (2018).
120. Neele, A. E., Van Den Bossche, J., Hoeksema, M. A. & De Winther, M. P. J. Epigenetic pathways in macrophages emerge as novel targets in atherosclerosis. *Eur. J. Pharmacol.* **763**, 79–89 (2015).
121. Wang, Z., Long, H., Chang, C., Zhao, M. & Lu, Q. Crosstalk between metabolism and epigenetic modifications in autoimmune diseases: a comprehensive overview. *Cell. Mol. Life Sci.* **75**, 3353–3369 (2018).
122. Van den Bossche, J. & Saraber, D. L. Metabolic regulation of macrophages in tissues. *Cell. Immunol.* **330**, 54–59 (2018).
123. Covarrubias, A. J. et al. Akt-mTORC1 signaling regulates Acly to integrate metabolic input to control of macrophage activation. *eLife* **5**, e11612 (2016).
124. Bottini, N. & Firestein, G. S. Epigenetics in rheumatoid arthritis: a primer for rheumatologists. *Curr. Rheumatol. Rep.* **15**, 372 (2013).
125. Bottini, N. & Firestein, G. S. Duality of fibroblast-like synoviocytes in RA: passive responders and imprinted aggressors. *Nat. Rev. Rheumatol.* **9**, 24–33 (2013).
126. Kuo, D. et al. HBEGF^{+} macrophages in rheumatoid arthritis induce fibroblast invasiveness. *Sci. Transl. Med.* **11**, eaau8587 (2019).
127. Ginhoux, F. et al. Fate mapping analysis reveals that adult microglia derive from primitive macrophages. *Science* **330**, 841–845 (2010).
128. Schulz, C. et al. A lineage of myeloid cells independent of Myb and hematopoietic stem cells. *Science* **335**, 86–90 (2012).
129. Yona, S. et al. Fate mapping reveals origins and dynamics of monocytes and tissue macrophages under homeostasis. *Immunity* **38**, 79–91 (2013).
130. Epelman, S. et al. Embryonic and adult-derived resident cardiac macrophages are maintained through distinct mechanisms at steady state and during inflammation. *Immunity* **40**, 91–104 (2014).
131. Baardman, J., Licht, I., De Winther, M. P. J. & Van Den Bossche, J. Metabolic-epigenetic crosstalk in macrophage activation. *Epigenomics* **7**, 1155–1164 (2015).
132. Italiani, P. & Boraschi, D. From monocytes to M 1/M2 macrophages: phenotypical vs. functional differentiation. *Front. Immunol.* **5**, 1–22 (2014).
133. Davies, L. C. et al. Peritoneal tissue-resident macrophages are metabolically poised to engage microbes using tissue-niche fuels. *Nat. Commun.* **8**, 1–15 (2017).
134. Ziegler-Heitbrock, L. et al. Nomenclature of monocytes and dendritic cells in blood. *Blood* **116**, e74–e80 (2010).
135. Shortman, K. & Liu, Y.-J. Mouse and human dendritic cell subtypes. *Nat. Rev. Immunol.* **2**, 151–161 (2002).
136. Segura, E. et al. Human inflammatory dendritic cells induce Th17 cell differentiation. *Immunity* **38**, 336–348 (2013).
137. Radford, K. J. et al. Phenotypic and transcriptomic analysis of peripheral blood plasmacytoid and conventional dendritic cells in early drug naïve rheumatoid arthritis. *Front. Immunol.* **9**, 755 (2018).
138. Canavan, M. et al. Enriched CD141^{+} DCs in the joint are transcriptionally distinct, activated, and contribute to joint pathogenesis. *JCI Insight* **3**, e95228 (2018).
139. Aggarwal, S., Ghilardi, N., Xie, M. H., de Sauvage, F. J. & Gurney, A. L. Interleukin-23 promotes a distinct CD4 T cell activation state characterized by the production of interleukin-17. *J. Biol. Chem.* **278**, 1910–1914 (2003).
140. Krawczyk, C. M. et al. Toll-like receptor-induced changes in glycolytic metabolism regulate dendritic cell activation. *Blood* **115**, 4742–4749 (2010).
141. Everts, B. et al. Commitment to glycolysis sustains survival of NO-producing inflammatory dendritic cells. *Blood* **120**, 1422–1431 (2012).
142. Everts, B. et al. TLR-driven early glycolytic reprogramming via the kinases TBK1-IKKε supports the anabolic demands of dendritic cell activation. *Nat. Immunol.* **15**, 323–332 (2014).
143. Jantsch, J. et al. Hypoxia and hypoxia-inducible factor-1α modulate lipopolysaccharide-induced dendritic cell activation and function. *J. Immunol.* **180**, 4697–4705 (2008).
144. Guak, H. et al. Glycolytic metabolism is essential for CCR7 oligomerization and dendritic cell migration. *Nat. Commun.* **9**, 1–12 (2018).
145. Pantel, A. et al. Direct type I IFN but not MDA5/TLR3 activation of dendritic cells is required for maturation and metabolic shift to glycolysis after poly IC stimulation. *PLoS Biol.* **12**, e1001759 (2014).
146. Okano, T. et al. 3-bromopyruvate ameliorate autoimmune arthritis by modulating Th17/Treg cell differentiation and suppressing dendritic cell activation. *Sci. Rep.* **7**, 42412 (2017).
147. Canavan, M. et al. Rheumatoid arthritis synovial microenvironment induces metabolic and functional adaptations in dendritic cells. *Clin. Exp. Immunol.* **202**, 226–238 (2020).
148. Millard, A. L. et al. Butyrate affects differentiation, maturation and function of human monocyte-derived dendritic cells and macrophages. *Clin. Exp. Immunol.* **130**, 245–255 (2002).
149. He, W. et al. Citric acid cycle intermediates as ligands for orphan G-protein-coupled receptors. *Nature* **429**, 188–193 (2004).
150. Rubic, T. et al. Triggering the succinate receptor GPR91 on dendritic cells enhances immunity. *Nat. Immunol.* **9**, 1261–1269 (2008).
151. Li, L. et al. Dendritic cells tolerized with adenosine A2AR agonist attenuate acute kidney injury. *J. Clin. Invest.* **122**, 3931–3942 (2012).
152. Saraiva, A. L. et al. Succinate receptor deficiency attenuates arthritis by reducing dendritic cell traffic and expansion of Th17 cells in the lymph nodes. *FASEB J.* **32**, 6550–6558 (2018).
153. Rivellese, F. et al. Persistence of mast cell-positive synovitis in early rheumatoid arthritis following treatment with conventional synthetic disease modifying anti-rheumatic drugs. *Front. Pharmacol.* **11**, 1051 (2020).
154. Yamin, R. et al. High percentages and activity of synovial fluid NK cells present in patients with advanced stage active Rheumatoid Arthritis. *Sci. Rep.* **9**, 1351 (2019).
155. Phong, B., Avery, L., Menk, A. V., Delgoffe, G. M. & Kane, L. P. Cutting edge: murine mast cells rapidly modulate metabolic pathways essential for distinct effector functions. *J. Immunol.* **198**, 640–644 (2017).
156. Rauber, S. et al. Resolution of inflammation by interleukin-9-producing type 2 innate lymphoid cells. *Nat. Med.* **23**, 938–944 (2017).
157. Surace, L. et al. Dichotomous metabolic networks govern human ILC2 proliferation and function. *Nat. Immunol.* **22**, 1367–1374 (2021).
158. Huang, N. & Perl, A. Metabolism as a target for modulation in autoimmune diseases. *Trends Immunol.* **39**, 562–576 (2018).
159. Menk, A. V. et al. Early TCR signaling induces rapid aerobic glycolysis enabling distinct acute T cell effector functions. *Cell Rep.* **22**, 1509–1521 (2018).
160. Yang, K. et al. T cell exit from quiescence and differentiation into Th2 cells depend on Raptor-mTORC1-mediated metabolic reprogramming. *Immunity* **39**, 1043–1056 (2013).
161. Capone, A. & Volpe, E. Transcriptional regulators of T helper 17 cell differentiation in health and autoimmune diseases. *Front. Immunol.* **11**, 348 (2020).
162. Michalek, R. D. et al. Cutting edge: distinct glycolytic and lipid oxidative metabolic programs are essential for effector and regulatory CD4^{+} T cell subsets. *J. Immunol.* **186**, 3299–3303 (2011).
163. Bettelli, E. et al. Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells. *Nature* **441**, 235–238 (2006).
164. Shin, B. et al. Mitochondrial oxidative phosphorylation regulates the fate decision between pathogenic Th17 and regulatory T cells. *Cell Rep.* **30**, 1898–1909.e4 (2020).
165. Karmaus, P. W. F. et al. Metabolic heterogeneity underlies reciprocal fates of TH17 cell stemness and plasticity. *Nature* **565**, 101–105 (2019).
166. Floudas, A. et al. ACPA status correlates with differential immune profile in patients with rheumatoid arthritis. *Cells* **10**, 647 (2021).
167. Basdeo, S. A. et al. Ex-Th17 (nonclassical Th1) cells are functionally distinct from classical Th1 and Th17 cells and are not constrained by regulatory T cells. *J. Immunol.* **198**, 2249–2259 (2017).
168. Yang, Z., Fujii, H., Mohan, S. V., Goronzy, J. J. & Weyand, C. M. Phosphofructokinase deficiency impairs ATP generation, autophagy, and redox balance in rheumatoid arthritis T cells. *J. Exp. Med.* **210**, 2119–2134 (2013).
169. Floudas, A. et al. Loss of balance between protective and pro-inflammatory synovial tissue T-cell polyfunctionality predates clinical onset of rheumatoid arthritis. *Ann. Rheum. Dis.* **81**, 193–205 (2021).
170. Li, Y. et al. The DNA repair nuclease MRE11A functions as a mitochondrial protector and prevents T cell pyroptosis and tissue inflammation. *Cell Metab.* **30**, 477–492.e6 (2019).
171. Weyand, C. M., Yang, Z. & Goronzy, J. J. T-cell aging in rheumatoid arthritis. *Curr. Opin. Rheumatol.* **26**, 93–100 (2014).
172. Son, H. J. et al. Metformin attenuates experimental autoimmune arthritis through reciprocal regulation of Th17/Treg balance and osteoclastogenesis. *Mediators Inflamm.* **2014**, 973986 (2014).
173. Wu, B. et al. Succinyl-CoA ligase deficiency in pro-inflammatory and tissue-invasive T cells. *Cell Metab.* **32**, 967–980 e5 (2020).
174. Wu, B. et al. Mitochondrial aspartate regulates TNF biogenesis and autoimmune tissue inflammation. *Nat. Immunol.* **22**, 1551–1562 (2021).
175. Egan, P. J., van Nieuwenhuijze, A., Campbell, I. K. & Wicks, I. P. Promotion of the local differentiation of murine Th17 cells by synovial macrophages during acute inflammatory arthritis. *Arthritis Rheum.* **58**, 3720–3729 (2008).
176. Nanki, T. et al. Pathogenic role of the CXCL16-CXCR6 pathway in rheumatoid arthritis. *Arthritis Rheum.* **52**, 3004–3014 (2005).
177. Ohradanova-Repic, A. et al. Extracellular purine metabolism is the switchboard of immunosuppressive macrophages and a novel target to treat diseases with macrophage imbalances. *Front. Immunol.* **9**, 852 (2018).
178. Vuononvirta, J., Marelli-Berg, F. M. & Poobalasingam, T. Metabolic regulation of T lymphocyte motility and migration. *Mol. Asp. Med.* **77**, 100888 (2021).
179. Neveu, M. A. et al. Lactate production precedes inflammatory cell recruitment in arthritic ankles: an imaging study. *Mol. Imaging Biol.* **22**, 1324–1332 (2020).
180. Nakaya, M. et al. Inflammatory T cell responses rely on amino acid transporter ASCT2 facilitation of glutamine uptake and mTORC1 kinase activation. *Immunity* **40**, 692–705 (2014).

181. Xu, Y. et al. Glycolysis determines dichotomous regulation of T cell subsets in hypoxia. *J. Clin. Invest.* **126**, 2678–2688 (2016).
182. Chen, P. M. et al. Kidney tissue hypoxia dictates T cell-mediated injury in murine lupus nephritis. *Sci. Transl. Med.* **12**, eaay1620 (2020).
183. Dang, E. V. et al. Control of T_H17/T_{reg} balance by hypoxia-inducible factor 1. *Cell* **146**, 772–784 (2011).
184. Zeng, Q. H. et al. B cells polarize pathogenic inflammatory T helper subsets through ICOSL-dependent glycolysis. *Sci. Adv.* **6**, eabb6296 (2020).
185. Akkaya, M. et al. Second signals rescue B cells from activation-induced mitochondrial dysfunction and death. *Nat. Immunol.* **19**, 871–884 (2018).
186. Luo, W., Weisel, F. & Shlomchik, M. J. B cell receptor and CD40 signaling are rewired for synergistic induction of the c-Myc transcription factor in germinal center B cells. *Immunity* **48**, 313–326.e5 (2018).
187. Kennedy, D. E. et al. Novel specialized cell state and spatial compartments within the germinal center. *Nat. Immunol.* **21**, 660–670 (2020).
188. Choi, S. C. & Morel, L. Immune metabolism regulation of the germinal center response. *Exp. Mol. Med.* **52**, 348–355 (2020).
189. Tsui, C. et al. Protein kinase C-β dictates B cell fate by regulating mitochondrial remodeling, metabolic reprogramming, and heme biosynthesis. *Immunity* **48**, 1144–1159.e5 (2018).
190. Gaudette, B. T., Jones, D. D., Bortnick, A., Argon, Y. & Allman, D. mTORC1 coordinates an immediate unfolded protein response-related transcriptome in activated B cells preceding antibody secretion. *Nat. Commun.* **11**, 723 (2020).
191. Jellusova, J. et al. Gsk3 is a metabolic checkpoint regulator in B cells. *Nat. Immunol.* **18**, 303–312 (2017).
192. Cho, S. H. et al. Germinal centre hypoxia and regulation of antibody qualities by a hypoxia response system. *Nature* **537**, 234–238 (2016).
193. Floudas, A. et al. Pathogenic, glycolytic PD-1$^+$ B cells accumulate in the hypoxic RA joint. *JCI Insight* **5**, e139032 (2020).
194. Guo, Y. et al. CD40L-dependent pathway is active at various stages of rheumatoid arthritis disease progression. *J. Immunol.* **198**, 4490–4501 (2017).
195. Corsiero, E., Nerviani, A., Bombardieri, M. & Pitzalis, C. Ectopic lymphoid structures: powerhouse of autoimmunity. *Front. Immunol.* **7**, 430 (2016).
196. Yanagihara, Y., Shiozawa, K., Takai, M., Kyogoku, M. & Shiozawa, S. Natural killer (NK) T cells are significantly decreased in the peripheral blood of patients with rheumatoid arthritis (RA). *Clin. Exp. Immunol.* **118**, 131–136 (1999).
197. Linsen, L. et al. Peripheral blood but not synovial fluid natural killer T cells are biased towards a Th1-like phenotype in rheumatoid arthritis. *Arthritis Res. Ther.* **7**, R493–R502 (2005).
198. Koppejan, H. et al. Altered composition and phenotype of mucosal-associated invariant T cells in early untreated rheumatoid arthritis. *Arthritis Res. Ther.* **21**, 3 (2019).
199. Zhao, M. et al. Altered thymic differentiation and modulation of arthritis by invariant NKT cells expressing mutant ZAP70. *Nat. Commun.* **9**, 2627 (2018).
200. Kumar, A. et al. Enhanced oxidative phosphorylation in NKT cells is essential for their survival and function. *Proc. Natl Acad. Sci. USA* **116**, 7439–7448 (2019).
201. Certo, M., Tsai, C. H., Pucino, V., Ho, P. C. & Mauro, C. Lactate modulation of immune responses in inflammatory versus tumour microenvironments. *Nat. Rev. Immunol.* **21**, 151–161 (2021).
202. Amedei, A. et al. Ex vivo analysis of pancreatic cancer-infiltrating T lymphocytes reveals that ENO-specific Tregs accumulate in tumor tissue and inhibit Th1/Th17 effector cell functions. *Cancer Immunol. Immunother.* **62**, 1249–1260 (2013).
203. Clementi, E., Brown, G. C., Feelisch, M. & Moncada, S. Persistent inhibition of cell respiration by nitric oxide: crucial role of S-nitrosylation of mitochondrial complex I and protective action of glutathione. *Proc. Natl Acad. Sci. USA* **95**, 7631–7636 (1998).
204. Geiger, R. et al. L-Arginine modulates T cell metabolism and enhances survival and anti-tumor activity. *Cell* **167**, 829–842.e13 (2016).
205. Niedbala, W., Cai, B. & Liew, F. Y. Role of nitric oxide in the regulation of T cell functions. *Ann. Rheum. Dis.* **65** (Suppl. 3), iii37–iii40 (2006).
206. Lu, Y. et al. Glucocorticoid receptor promotes the function of myeloid-derived suppressor cells by suppressing HIF1α-dependent glycolysis. *Cell. Mol. Immunol.* **15**, 618–629 (2018).
207. Moreno-Aurioles, V. R. & Sobrino, F. Glucocorticoids inhibit fructose 2,6-bisphosphate synthesis in rat thymocytes. Opposite effect of cycloheximide. *Biochim. Biophys. Acta* **1091**, 96–100 (1991).
208. Swerdlow, S. et al. Apoptosis inhibition by Bcl-2 gives way to autophagy in glucocorticoid-treated lymphocytes. *Autophagy* **4**, 612–620 (2008).
209. Gosselt, H. R. et al. Identification of metabolic biomarkers in relation to methotrexate response in early rheumatoid arthritis. *J. Pers. Med.* **10**, 271 (2020).
210. Falconer, J. et al. Spontaneously resolving joint inflammation is characterised by metabolic agility of fibroblast-like synoviocytes. *Front. Immunol.* **12**, 725641 (2021).
211. Angiari, S. et al. Pharmacological activation of pyruvate kinase M2 inhibits CD4$^+$ T cell pathogenicity and suppresses autoimmunity. *Cell Metab.* **31**, 391–405.e8 (2020).
212. Damasceno, L. E. A. et al. PKM2 promotes Th17 cell differentiation and autoimmune inflammation by fine-tuning STAT3 activation. *J. Exp. Med.* **217**, e20190613 (2020).
213. Xu, J. et al. Upregulated PKM2 in macrophages exacerbates experimental arthritis via STAT1 signaling. *J. Immunol.* **205**, 181–192 (2020).
214. Yang, X. et al. Pyruvate kinase M2 modulates the glycolysis of chondrocyte and extracellular matrix in osteoarthritis. *DNA Cell Biol.* **37**, 271–277 (2018).
215. Takahashi, S. et al. Glutaminase 1 plays a key role in the cell growth of fibroblast-like synoviocytes in rheumatoid arthritis. *Arthritis Res. Ther.* **19**, 1–10 (2017).
216. Li, H. M. et al. Inhibition of glycolysis by targeting lactate dehydrogenase A facilitates hyaluronan synthase 2 synthesis in synovial fibroblasts of temporomandibular joint osteoarthritis. *Bone* **141**, 115584 (2020).
217. Angelin, A. et al. Foxp3 reprograms T cell metabolism to function in low-glucose, high-lactate environments. *Cell Metab.* **25**, 1282–1293. e7 (2017).
218. Szczuka, I., Gamian, A. & Terlecki, G. 3-Bromopyruvate as a potential pharmaceutical in the light of experimental data. *Postepy Hig. Med. Dosw.* **71**, 988–996 (2017).
219. Zou, Y. et al. Inhibition of 6-phosphofructo-2-kinase suppresses fibroblast-like synoviocytes-mediated synovial inflammation and joint destruction in rheumatoid arthritis. *Br. J. Pharmacol.* **174**, 893–908 (2017).
220. Song, G. et al. Inhibition of hexokinases holds potential as treatment strategy for rheumatoid arthritis. *Arthritis Res. Ther.* **21**, 87 (2019).
221. Veras, F. P. et al. Fructose 1,6-bisphosphate, a high-energy intermediate of glycolysis, attenuates experimental arthritis by activating anti-inflammatory adenosinergic pathway. *Sci. Rep.* **5**, 15171 (2015).
222. Yan, H., Zhou, H. F., Hu, Y. & Pham, C. T. Suppression of experimental arthritis through AMP-activated protein kinase activation and autophagy modulation. *J. Rheum. Dis. Treat.* **1**, 5 (2015).
223. Abdallah, M. S. et al. The AMPK modulator metformin as adjunct to methotrexate in patients with rheumatoid arthritis: a proof-of-concept, randomized, double-blind, placebo-controlled trial. *Int. Immunopharmacol.* **95**, 107575 (2021).
224. Kim, E. K. et al. Metformin rescues rapamycin-induced mitochondrial dysfunction and attenuates rheumatoid arthritis with metabolic syndrome. *Arthritis Res. Ther.* **22**, 77 (2020).
225. Chang, H. H. et al. A molecular signature of preclinical rheumatoid arthritis triggered by dysregulated PTPN22. *JCI Insight* **1**, e90045 (2016).
226. Dong, X. et al. ACPAs promote IL-1beta production in rheumatoid arthritis by activating the NLRP3 inflammasome. *Cell Mol. Immunol.* **17**, 261–271 (2020).
227. Yang, Z. et al. Restoring oxidant signaling suppresses proarthritogenic T cell effector functions in rheumatoid arthritis. *Sci. Transl. Med.* **8**, 331ra338 (2016).
228. Rooney, C. M., Mankia, K. & Emery, P. The role of the microbiome in driving RA-related autoimmunity. *Front. Cell Dev. Biol.* **8**, 538130 (2020).
229. Courbon, G. et al. *Porphyromonas gingivalis* experimentally induces periodontis and an anti-CCP2-associated arthritis in the rat. *Ann. Rheum. Dis.* **78**, 594–599 (2019).
230. Konig, M. F. et al. Aggregatibacter actinomycetemcomitans-induced hypercitrullination links periodontal infection to autoimmunity in rheumatoid arthritis. *Sci. Transl. Med.* **8**, 369ra176 (2016).
231. Maurice, M. M. et al. Expression of the thioredoxin-thioredoxin reductase system in the inflamed joints of patients with rheumatoid arthritis. *Arthritis Rheum.* **42**, 2430–2439 (1999).

Author contributions
All authors contributed equally to all aspects of the article.

Competing interests
The authors declare no competing interests.

Peer review information
Nature Reviews Rheumatology thanks C. Mauro (who co-reviewed with M. Certo), M. Guma and G. Tsokos for their contribution to the peer review of this work.

Mechanisms of joint destruction in rheumatoid arthritis — immune cell–fibroblast–bone interactions

Noriko Komatsu and Hiroshi Takayanagi ✉

Abstract | Rheumatoid arthritis (RA) is characterized by inflammation and destruction of bone and cartilage in affected joints. Autoimmune responses lead to increased osteoclastic bone resorption and impaired osteoblastic bone formation, the imbalance of which underlies bone loss in RA, which includes bone erosion, periarticular bone loss and systemic osteoporosis. The crucial role of osteoclasts in bone erosion has been demonstrated in basic studies as well as by the clinical efficacy of antibodies targeting RANKL, an important mediator of osteoclastogenesis. Synovial fibroblasts contribute to joint damage by stimulating both pro-inflammatory and tissue-destructive pathways. New technologies, such as single-cell RNA sequencing, have revealed the heterogeneity of synovial fibroblasts and of immune cells including T cells and macrophages. To understand the mechanisms of bone damage in RA, it is important to clarify how the immune system promotes the tissue-destructive properties of synovial fibroblasts and influences bone cells. The interaction between immune cells and fibroblasts underlies the imbalance between regulatory T cells and T helper 17 cells, which in turn exacerbates not only inflammation but also bone destruction, mainly by promoting RANKL expression on synovial fibroblasts. An improved understanding of the immune mechanisms underlying joint damage and the interplay between the immune system, synovial fibroblasts and bone will contribute to the identification of novel therapeutic targets in RA.

Rheumatoid arthritis (RA) is an autoimmune disease caused by a complex interaction between genetic and environmental factors, and possibly consists of aetiologically heterogeneous subpopulations[1,2]. Autoimmunity is the first step in the pathogenesis of RA and a high serum concentration of autoantibodies, such as anti-citrullinated peptide antibodies (ACPAs), is a hallmark of RA, although some patients are seronegative[1,2]. Immune cells, such as activated T cells, B cells and macrophages, produce pro-inflammatory cytokines and stimulate synovial fibroblasts (tissue-resident mesenchymal cells in the joints) to polarize into pro-inflammatory and tissue-destructive subsets[3–5]. Tissue-destructive synovial fibroblasts express receptor activator of NF-κB ligand (RANKL) and induce osteoclasts to promote bone destruction, and express matrix metalloproteinases (MMPs) that accelerate cartilage degradation (described in detail in BOX 1)[3–6]. Current therapies for RA are not effective in all patients and are associated with adverse effects such as infection; therefore, it is necessary to develop new therapies, ideally targeting joint-specific pathogenic molecules or cells. Moreover, as the existing therapies for RA are not capable of completely preventing structural damage or restoring joints, the time has come to thoroughly elucidate the immune mechanisms of joint destruction in RA in order to establish the scientific basis for novel therapeutic approaches. To that end, it is important to recognize the importance of the interactions among the immune system, fibroblasts and bone.

Extending the concept of immune cell–fibroblast and immune cell–bone interactions, we herein provide an overview of recent advances in these areas and explore the interactivity within the immune cell–fibroblast–bone triad[7]. We provide an overview of the relevant immune mechanisms and mechanisms of structural damage (including bone erosion and periarticular and systemic bone loss), summarize the interplay among immune cells, fibroblasts and bone in both active disease and remission, and provide a perspective on current and future strategies for the treatment of structural damage in RA.

Department of Immunology, Graduate School of Medicine and Faculty of Medicine, The University of Tokyo, Tokyo, Japan.

✉*e-mail: takayana@m.u-tokyo.ac.jp*

https://doi.org/10.1038/s41584-022-00793-5

Overview of joint structure and biology

The synovium consists of two layers, an intimal lining layer and a sublining layer. In a healthy joint, the lining layer, which consists of resident macrophages and lining

Key points

- T helper 17 (T_H17) cells and autoantibodies promote inflammation and tissue destruction in rheumatoid arthritis (RA) by activating other immune cells and synovial fibroblasts, leading to synovitis, bone erosion and cartilage damage.
- Synovial fibroblasts in RA comprise pro-inflammatory and tissue-destructive subsets, the latter of which express RANKL and matrix metalloproteinases that are involved in osteoclastic bone resorption and cartilage degradation, respectively.
- Bone lesions in RA are classified as bone erosion, periarticular bone loss and systemic osteoporosis, which are induced by distinct mechanisms.
- The integration of data from single-cell RNA sequencing and biological studies provides a detailed depiction of the interplay among immune cells, fibroblasts and bone in RA pathogenesis.
- Therapeutic strategies to modulate pathogenic synovial fibroblasts and to achieve a balance between regulatory T cells and T_H17 cells and/or between bone resorption and repair will help achieve structural remission.

synovial fibroblasts, forms a thin barrier at the interface between the sublining and the synovial fluid space. The sublining layer contains endothelial cells and sublining synovial fibroblasts. Synovial fibroblasts produce matrix proteins such as collagen to maintain the structure of the synovium, and lubricate and nourish cartilage surfaces by producing hyaluronic acid and other joint lubricants such as lubricin.

Bone homeostasis is maintained by a balance between bone resorption by osteoclasts and bone formation by osteoblasts[3,8]. Osteoclasts are the exclusive bone-resorbing cells and differentiate from bone marrow-derived monocyte–macrophage lineage cells. Osteoclasts resorb bone via decalcification and matrix degradation that are mediated by the secretion of hydrogen ions and matrix-degrading enzymes, respectively. Bone-forming osteoblasts, which produce bone matrix proteins and mediate mineralization, are of mesenchymal origin[3,8] Some osteoblasts become embedded in the bone matrix where they differentiate into osteocytes, which are thought to orchestrate both osteoclastic bone resorption and osteoblastic bone formation in response to mechanical stress and hormonal cues[3,9,10].

RANKL, a TNF family cytokine, and macrophage colony-stimulating factor (M-CSF) are essential molecules for osteoclastogenesis[3,8,11]. RANKL binds to its receptor RANK and activates downstream signalling pathways such as NF-κB and AP-1, leading to the autoamplification of NFATc1, the master regulator of osteoclastogenesis[12]. Osteoblasts and osteocytes express RANKL and stimulate osteoclastogenesis necessary for the renewal of bone under physiological conditions[3,9,10]. Osteoprotegerin, a decoy receptor for RANKL, inhibits the RANK–RANKL interaction[13]. M-CSF promotes proliferation of osteoclast precursors and activation and survival of osteoclasts[3,8]. Osteoblast differentiation is stimulated by osteogenic cytokines such as Wnt and bone morphogenetic protein (BMP). Sclerostin, an inhibitor of Wnt signalling, is mainly produced by osteocytes. Mechanical loading decreases sclerostin expression in osteocytes and promotes bone formation, whereas mechanical unloading increases RANKL expression in osteocytes and promotes osteoclastogenesis[10,14,15]. Activation of the immune system in autoimmune diseases disturbs bone homeostasis by acting directly on bone cells or by stimulating joint-resident cells such as fibroblasts, as discussed below.

Immune mechanisms in RA

In RA, the immune system stimulates synovial fibroblasts to exert inflammatory and tissue-destructive effects and exacerbate RA pathogenesis[4]. Fibroblasts are the most abundant mesenchymal stromal cells and serve as structural cells that define the architecture of organs; however, attention has increasingly been given to the role of fibroblasts in the pathogenesis of fibrosis, cancer and autoimmunity[16,17]. New technologies, including single-cell RNA sequencing (scRNA-seq) and mass cytometry, have revealed the heterogeneity of synovial fibroblasts and enabled the identification of functionally and phenotypically distinct pro-inflammatory and tissue-destructive subsets of these cells in RA[4,5,18]. Herein we describe the autoimmune responses and the subsequent activation of the distinct fibroblast subsets that mediate inflammation and structural damage in RA.

Immune response activation

Genome-wide association studies have revealed strong genetic associations between RA and the HLA regions, indicating the importance of antigen recognition in RA pathogenesis[1,2]. The citrullination of peptides is mediated by the peptidylarginine deiminases that are upregulated by smoking and periodontitis, suggesting a link between environmental risk factors and RA pathogenesis[1,2]. The combination of genetic and environmental factors contributes to the breakdown of self-tolerance and the onset of autoimmune arthritis.

The cascade of autoimmune responses starts with T cells recognizing self antigens presented by antigen-presenting cells, such as dendritic cells. $CD4^+$ T cells differentiate into T helper (T_H) cells, among which T_H17 cells have a critical role in autoimmune inflammation[3,7]. T follicular helper cells ($CXCR5^+PD\text{-}1^{hi}$), which reside in lymph nodes, as well as newly identified T peripheral helper (T_{PH}) cells ($CXCR5^-PD\text{-}1^{hi}$) that reside in the inflamed synovium, help B cells to produce autoantibodies such as ACPAs and rheumatoid factor[19]. T_H17-derived IL-17 and other cytokines (such as IL-21, IL-22 and TNF) mediate the proliferation of synovial fibroblasts as well as innate immune cells, including neutrophils and macrophages, and induce the expression of pro-inflammatory cytokines (such as TNF, IL-6 and IL-1) and chemokines (such as CCL20 and CCL2) by these cells[3,7]. T_H17 cells also increase the pro-inflammatory activity of autoantibodies via the desialylation of autoantibodies in an IL-21-dependent and IL-22-dependent manner[20]. A decrease in IgG glycosylation in $ACPA^+$ asymptomatic individuals not only parallels the clinical onset of RA, but is also associated with disease activity in $ACPA^+$ patients with RA, suggesting the importance of T_H17 cells and autoantibodies in the immune activation phase of RA[20,21]. A study published in 2021 showed that tissue-resident memory $CD8^+$ T cells cause arthritis flares[22]. Immune complexes activate innate immune cells to further upregulate pro-inflammatory cytokines and chemokines[3,7]. Synovial fibroblasts amplify inflammation in response to these inflammatory mediators as well

as mechanical strain[23]. According to a 2019 scRNA-seq study, IL-6 is produced mainly by synovial fibroblasts, and macrophages are the main producers of IL-1 and TNF; T cells and B cells have also been found to produce TNF in RA[18] (FIG. 1). Another scRNA-seq study revealed that CCL13, CCL18 and MMP3 are upregulated in synovial myeloid cell subsets in ACPA$^-$ RA as compared with ACPA$^+$ RA, which could explain, at least in part, the difference in immune mechanisms between seronegative and seropositive RA[24].

Fibroblast activation

Under arthritic conditions, synovial fibroblasts acquire an aggressive (activated, proliferative and invasive) phenotype and are important in the pathogenesis of RA[17]. This aggressive phenotype of RA synovial fibroblasts is seemingly induced by the inflammatory milieu in the synovium. Analysis of DNA promoter methylation in synovial fibroblasts revealed that the DNA methylation pattern in synovial fibroblasts from patients with very early RA is already different from that in synovial fibroblasts under healthy conditions, suggesting that epigenetic modification is not just a consequence of inflammation, but could also be a cause of disease initiation and progression[25].

Metabolic changes, particularly an increase in glycolysis in synovial fibroblasts, are also linked with the aggressive phenotype of synovial fibroblasts in arthritis[17]. Glycolysis is the source of ATP under hypoxic conditions (which is considered to be a feature of the synovial microenvironment in RA), although this metabolic pathway is less efficient than oxidative phosphorylation. The expression of hypoxia-inducible factor 1α (HIF1α), an inducer of glycolysis, is linked to the aggressive features of synovial fibroblasts[26]. In addition, activation of intracellular complement C3 and C3a receptor on synovial fibroblasts after repeated inflammatory challenges induces metabolic reprogramming that promotes the activation of these cells and the priming of synovial tissue for inflammation[27]. Although these findings suggest that tissue priming occurs independently of adaptive immunity, the efficacy of abatacept (CTLA4-immunoglobulin (Ig), which prevents T cell costimulation) in RA suggests that activation of T cells might be necessary even in the chronic phase of the disease and that the reprogramming of synovial fibroblasts by repetitive priming might require T cell help. Considering that synovial fibroblasts are functionally heterogeneous, as we discuss below, it will be important to clarify how the polarization of fibroblasts into distinct synovial fibroblast subsets with inflammatory or tissue-destructive properties is determined.

Fibroblast heterogeneity

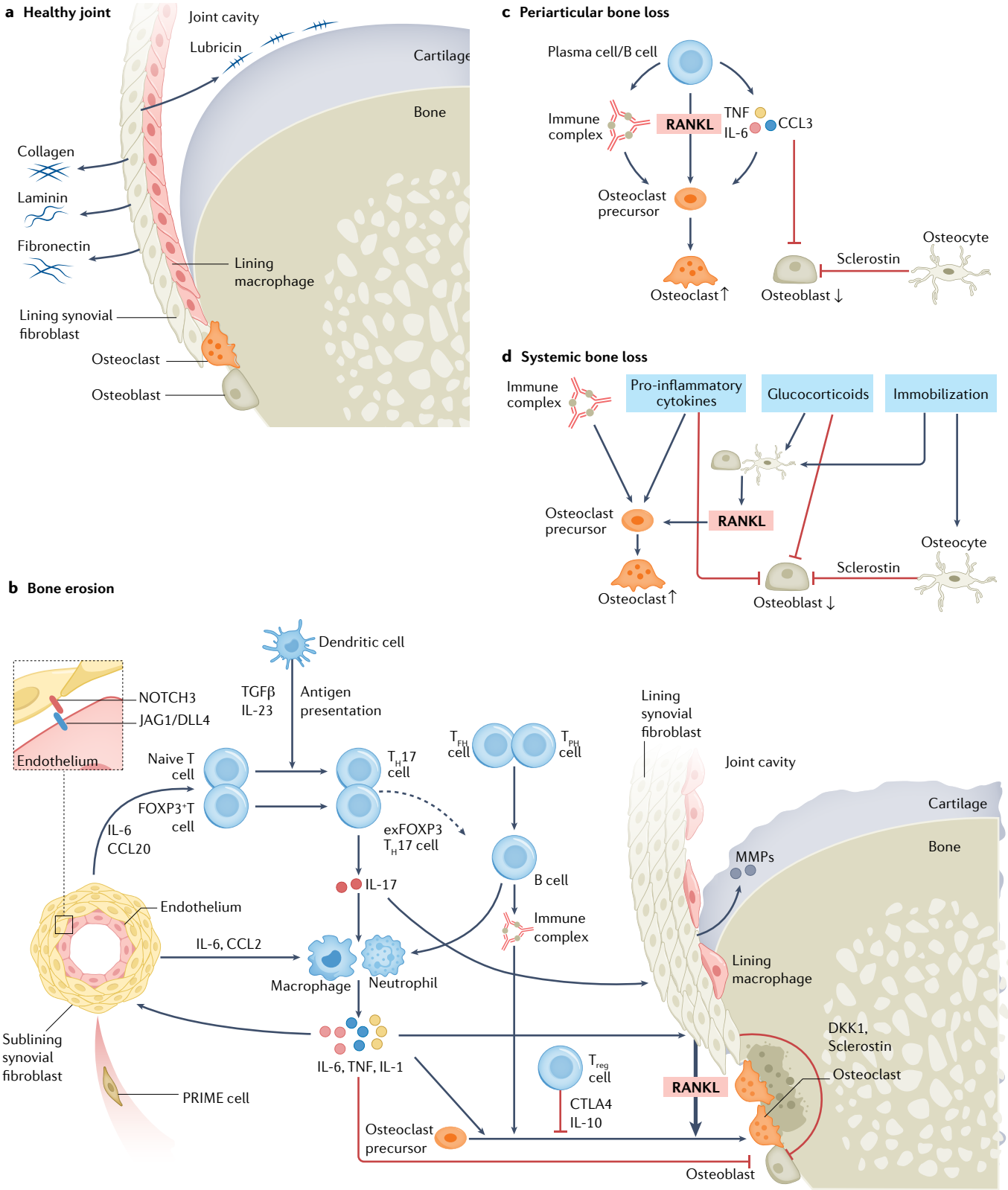
The phenotypic and functional heterogeneity of synovial fibroblasts is attracting increasing attention. Cadherin-11 is one of the most frequently investigated synovial fibroblast surface markers and has been linked to synovial fibroblast activation and inflammation. Cadherin-11 is expressed mainly in lining synovial fibroblasts, although it is also expressed in certain sublining synovial fibroblasts[28]. Cadherin-11 activates mitogen-activated protein kinases and NF-κB, which induce synovial fibroblasts to secrete pro-inflammatory cytokines such as IL-6 (REF.[29]). *Cdh11*-deficient mice have a hypoplastic synovial lining and exhibit reduced inflammation and cartilage erosion in the serum transfer-induced arthritis model[28].

scRNA-seq studies published in the past few years have identified synovial fibroblast subsets with inflammatory and tissue-destructive properties both in mouse and human arthritis[4,5,18]. Application of scRNA-seq to RA synovial fibroblasts characterized by the expression of podoplanin (PDPN) identified three major subpopulations: CD34$^-$THY1$^-$, CD34$^-$THY1$^+$ and CD34$^+$ cells. Among these subpopulations, the CD34$^-$THY1$^+$ cells, which localize to the perivascular zone in the inflamed synovium, are highly proliferative and secrete pro-inflammatory cytokines[5]. Subsequently, the integration of single-cell transcriptomics and mass cytometry revealed that sublining CD34$^-$CD90$^+$HLA-DR^{hi} synovial fibroblasts are expanded in the RA synovium and constitute a major source of IL-6 and CXCL12 (also known as stromal cell-derived factor 1)[18]. Moreover, a separate single-cell transcriptional analysis found two distinct subpopulations of fibroblast activation protein-α (FAPα)-expressing fibroblasts: inflammatory FAPα$^+$THY1$^+$ cells, located in the sublining, and tissue-destructive FAPα$^+$THY1$^-$ cells, located in the synovial lining layer[4]. Notably, in mice with serum transfer-induced arthritis, adoptive transfer of the FAPα$^+$THY1$^+$ synovial fibroblasts subset induces inflammation, whereas transfer of the FAPα$^+$THY1$^-$ subset induces joint destruction[4].

Box 1 | Cartilage degradation

Cartilage plays an important role in tissue patterning, skeletal development and joint movement[151,152]. Cartilage lacks blood and lymph vessels, and is composed of chondrocytes and extracellular matrix components such as type 2 collagen and aggrecan. Blockade of receptor activator of NF-κB ligand (RANKL) is unable to block cartilage damage in rheumatoid arthritis (RA), suggesting that cartilage erosion is independent of RANKL[126]. Cartilage degradation in RA results from excessive immune responses. Pro-inflammatory cytokines, including IL-1, TNF, IL-6 and IL-17, induce the production of cartilage-degrading enzymes such as matrix metalloproteinases (MMPs) as well as aggrecanases (such as ADAMTS-4 and ADAMTS-5) in synovial fibroblasts and inhibit the production of extracellular matrix by chondrocytes[151,152]. Among the MMPs, MMP1, MMP3, MMP9, MMP13 and MMP14 are upregulated in RA synovial fibroblasts. MMP14 (also known as MT1-MMP) is considered to be important in RA owing to its high level of expression in the RA synovium, following the results of functional analyses, and because treatment with an anti-MMP14 antibody suppressed cartilage destruction in a mouse model of RA[151,152]. MMP3 has been shown to be a useful marker of disease activity and predictor of the progression of cartilage destruction[153]. It is likely that the level of MMP3 reflects the activation state of synovial fibroblasts involved in tissue destruction.

Osteoarthritis (OA), which is the most prevalent age-related cartilage disorder, is caused by a combination of genetic background and environmental factors (such as excessive mechanical stress)[151,152]. The major difference between RA and OA is the source of the enzymes involved: in OA, chondrocytes mainly produce MMP and ADAMTS families, whereas activated synovial fibroblasts are sufficient for joint damage in RA. Nonetheless, shared mechanisms might underlie both OA and RA, as inflammation is also suggested to be involved in OA pathogenesis. For example, gremlin-1, an antagonist of bone morphogenetic protein signalling that is induced by mechanical loading, accelerates the induction of catabolic enzymes (MMPs and ADAMTS-5), inhibits type 2 collagen and aggrecan in OA, and is highly expressed in the RA synovium[154,155]. Thus, studies in OA are likely to contribute to a better understanding of the mechanisms underlying cartilage destruction in RA.



What is the mechanism underlying the generation of inflammatory or tissue-destructive synovial fibroblasts? Activation of Notch signalling induces the production of pro-inflammatory cytokines from RA synovial fibroblasts. In a 2020 study, scRNA-seq revealed that the interaction of Notch ligands expressed by endothelial cells with the receptor NOTCH3 on sublining synovial fibroblasts activates Notch signalling and drives the polarization of THY1$^+$ inflammatory synovial fibroblasts[30]. The genetic deletion of *Notch3* or the blockade of NOTCH3 signalling attenuates the inflammation and bone destruction in

Fig. 1 | **Mechanism of structural damage in rheumatoid arthritis. a** | Under physiological conditions, synovial fibroblasts lubricate and nourish the cartilage surface by producing hyaluronic acid and other joint lubricants such as lubricin. **b** | In rheumatoid arthritis, dendritic cells present autoantigens and produce cytokines that induce the differentiation of naive $CD4^+$ T cells into T helper (T_H) cells such as T_H17 cells, T follicular helper (T_{FH}) cells and T peripheral helper (T_{PH}) cells. The conversion of $FOXP3^+$ T cells to exFOXP3T_H17 cells is promoted by IL-6 produced by synovial fibroblasts. IL-17 activates sublining synovial fibroblasts, macrophages and neutrophils, and induces the expression of pro-inflammatory cytokines and chemokines from these cells. T_{FH} and T_{PH} cells help B cells to produce autoantibodies and immune complexes. T_H17 cells upregulate the activity of autoantibodies by regulating antibody glycosylation. Immune complexes activate innate immune cells to further upregulate pro-inflammatory cytokines and chemokines. Pro-inflammatory cytokines upregulate expression of receptor activator of NF-κB ligand (RANKL) in synovial fibroblasts. Pro-inflammatory cytokines and IgG immune complexes directly promote differentiation of osteoclasts through Fc receptors. IL-17 activates lining synovial fibroblasts to express RANKL and matrix metalloproteinases (MMPs), which induce osteoclastogenesis and cartilage degradation. Pro-inflammatory cytokines as well as Wnt inhibitors (such as DKK1 and sclerostin) inhibit osteoblastic bone formation. Regulatory T (T_{reg}) cells inhibit osteoclastogenesis. **c** | Periarticular bone loss. Plasma cells potently induce periarticular bone loss by expressing RANKL, autoantibodies and pro-inflammatory cytokines. B cells inhibit osteoblastic bone formation via TNF, CCL3 and IL-6. **d** | Systemic bone loss. Pro-inflammatory cytokines and immune complexes that circulate from inflamed joints as well as glucocorticoid administration promote osteoclastogenesis and inhibit osteoblastogenesis. Reduced mechanical loading induces expression of sclerostin and RANKL by osteocytes, leading to inhibition of osteoblastogenesis and stimulation of osteoclastogenesis, respectively. PRIME cell, pre-inflammatory mesenchymal cell.

serum transfer-induced arthritis[30]. TNF signalling is also important for the polarization of inflammatory synovial fibroblasts, as the activation of TNF signalling exclusively in fibroblasts induces arthritis in mice[31,32]. Moreover, the IL-6 family cytokine leukaemia inhibitory factor (LIF), which is upregulated in synovial fibroblasts under arthritic conditions, acts in an autocrine manner via LIF receptor to promote STAT4 activation, which increases production of important pro-inflammatory factors, including IL-6, leading to the polarization of inflammatory synovial fibroblasts[33].

Although inflammation is known to also promote tissue-destructive synovial fibroblasts, the stimulatory factors, intracellular signal transduction pathways and transcriptional machinery underlying the generation of these cells remain to be elucidated. The most important feature of tissue-destructive synovial fibroblasts is the production of RANKL, but they also produce tissue-destructive factors such as MMPs, thus orchestrating a variety of mechanisms that are involved in bone and cartilage destruction[4,5]. Understanding how RANKL production is induced in synovial fibroblasts will be helpful for determining the main mechanisms driving these cells.

Whether these distinct populations of synovial fibroblasts are subsets with fixed phenotypes or whether they have phenotypic plasticity also remains unclear. Fate mapping and/or tracing experiments in vivo are necessary to address this question.

Structural damage in RA

Structural abnormalities in RA involve bone erosion as well as periarticular and systemic bone loss. Osteoclasts were first observed at the interface between the inflamed synovium and bone in the 1980s, although it was initially unclear why autoimmunity increased osteoclastic bone resorption[34]. The generation of osteoclasts by culturing synovial cells from patients with RA indicated that both osteoclast precursor cells and osteoclastogenesis-supporting fibroblasts were present in the RA synovium[35]. Moreover, synovial fibroblasts were found to express a high level of RANKL, a molecule that is important for osteoclast differentiation[36,37]. This finding suggested that the immune system induced osteoclastogenesis mainly by stimulating synovial fibroblasts, rather than by acting directly on osteoclast precursor cells, and indicated the importance of tissue-destructive fibroblasts in arthritis.

Bone erosion

How does inflammation cause bone erosion? Protein-degrading enzymes were originally thought to sufficiently explain the bone erosion that occurs in arthritis; however, the importance of osteoclast-mediated bone resorption was suggested by the essential role of these cells in bone resorption in physiological bone remodelling and by the observation that osteoclasts are numerous at the synovium–bone interface in RA. Indeed, mice lacking osteoclasts are protected from bone erosion in TNF-transgenic (TNF-Tg) and serum transfer-induced models of arthritis, indicating the primacy of osteoclasts in bone erosion in RA[38,39]. The increased osteoclastogenesis observed in arthritis is attributed mainly to the increased expression of RANKL, as RANKL-deficient mice exhibit much less severe bone damage in arthritis than their matched littermates[38].

Synovial fibroblasts, as well as T cells and B cells, express RANKL when activated[36,37,40–43]. A long-standing question was which cell type induces osteoclastogenesis in the inflamed synovium. In the collagen-induced arthritis (CIA) model, mice lacking RANKL expression in synovial fibroblasts, but not those with T cell-specific or B cell-specific RANKL deficiency, are protected from bone erosion, indicating that synovial fibroblasts are the primary RANKL-producing cells in the synovium in autoimmune arthritis[6,44]. These findings lend support to the concept of 'tissue-destructive fibroblasts'[6,44].

Pro-inflammatory cytokines such as TNF, IL-6 and IL-1, which are abundant in the synovium and synovial fluid in RA, promote RANKL expression by synovial fibroblasts. In addition, the immune system enhances osteoclastogenesis by activating osteoclast precursor cells in several ways. Pro-inflammatory cytokines act directly on osteoclast precursor cells to enhance signalling downstream of RANK as well as to increase the expression of co-stimulatory receptors for RANK[45–47]. Moreover, IgG immune complexes directly promote osteoclast differentiation through Fc receptors[48] (FIG. 1). Antibodies have been shown to stimulate the production of IL-8 and TNF as well as to promote osteoclastogenesis[49,50]. Serum concentration of soluble RANKL is associated with disease activity in RA[51]. However, studies in mice selectively lacking soluble RANKL have demonstrated that soluble RANKL does not contribute to physiological bone remodelling or to a model of postmenopausal osteoporosis[52]. The question of how soluble and membrane-bound RANKL contribute to bone

destruction in RA will be an interesting issue to explore in future investigations.

How are T cells involved in bone erosion in RA? Activated T cells express not only RANKL but also effector cytokines with either stimulatory or inhibitory effects on osteoclastogenesis[40,41]. T_H1 and T_H2 cells inhibit osteoclastogenesis through the expression of IFNγ and IL-4, respectively. T_H17 cells comprise an exclusively osteoclastogenic T cell subset that induces RANKL expression on synovial fibroblasts via production of IL-17, IL-21 and IL-22 (REFS[7,53,54]). Pro-inflammatory cytokines from IL-17-activated innate immune cells further induce RANKL expression on synovial fibroblasts and act on osteoclast precursor cells to activate the downstream pathway of RANK[3,7]. Desialylation of IgG by T_H17 cells also increases the osteoclastogenic capacity of immune complexes[20,55] (FIG. 1).

Regulatory T (T_{reg}) cells are pivotal in the suppression of immune responses and the prevention of autoimmunity[56]. FOXP3 functions as the master transcription factor for the development and function of T_{reg} cells[57]. Humans and mice deficient in FOXP3 exhibit lethal autoimmune diseases owing to a lack of T_{reg} cells[56]. Evidence from a combination of genome-wide association studies with epigenetic analysis or expression quantitative trait locus analysis suggests that T_{reg} cells are strongly associated with RA[58,59]. T_{reg} cells express high amounts of CTLA4 and IL-10, which act on osteoclast precursor cells and inhibit osteoclastogenesis[60,61]. Thus, T_{reg} cells not only regulate inflammation in arthritis, but also directly inhibit bone destruction. Considering that T_{reg} cells recognize autoantigens, the loss of FOXP3 expression in T_{reg} cells could exacerbate autoimmune arthritis. Indeed, some $FOXP3^+$ T cells are plastic, and they lose FOXP3 expression and convert to arthritogenic T_H17 cells, which exacerbate autoimmune arthritis[62]. These T_H17 cells of $FOXP3^+$ T cell origin (called exFOXP3T_H17 cells) induce osteoclastogenesis more efficiently than T_H17 cells derived from naive $CD4^+$ T cells. exFOXP3T_H17 cells produce copious amounts of effector molecules such as IL-17, CCR6, CCL20 and RANKL, and induce RANKL expression on synovial fibroblasts[62,63]. Thus, exFOXP3T_H17 cells are the osteoclastogenic T cell subset that most potently induces bone erosion.

How is osteoblastic bone formation impaired in RA? In arthritic joints, osteoblast function is impaired, especially in the bone adjacent to the inflammatory synovium[64]. Pro-inflammatory cytokines inhibit osteoblastic bone formation via several mechanisms[8].

TNF suppresses osteoblast differentiation by suppressing expression of the transcription factor RUNX2 and by upregulating inhibitors of Wnt signalling[65]. In the RA synovium, endogenous Wnt inhibitors such as Dickkopf-related protein 1 (DKK1), sclerostin, and Frizzled-related proteins are upregulated[8]; DKK1 and sclerostin inhibit Wnt signalling by binding to LRP5 and LRP6, which are receptors for canonical Wnt signalling, whereas Frizzled-related proteins bind directly to Wnt ligands. DKK1 is produced mainly by TNF-stimulated synovial fibroblasts. In patients with RA, variants of *Dkk1* variants are associated with severe joint destruction[66]. Sclerostin is produced not only by osteocytes, but also by TNF-stimulated synovial fibroblasts[67]. TNF also inhibits BMP signalling by inducing the production of BMP3, an endogenous BMP inhibitor, by osteoblasts[68].

IL-1 inhibits osteoblast differentiation, whereas IL-6 promotes osteoblast differentiation and bone formation under certain conditions[69,70]. Administration of IL-6 stimulates bone formation in mice via trans-signalling, but treatment with an antibody targeting IL-6 receptor has no negative effects on bone mass[71], possibly because IL-6 blockade has a positive influence on bone mass by suppressing inflammation mediated by IL-6 and other cytokines such as TNF as well as by inhibiting osteoclastogenic bone resorption.

The role of IL-17 in osteoblast differentiation is controversial[72]. Spondyloarthritis, including psoriatic arthritis and ankylosing spondylitis, is characterized by inflammation and new bone formation in entheses, both of which are known to be reduced by IL-17 blockade[73,74]. Conversely, IL-17 deficiency promotes bone formation without influencing inflammation and bone erosion in the joints of mice with serum transfer-induced arthritis[75]. IL-17 inhibits calvarial osteoblast differentiation in vitro by inducing osteoblast expression of secreted Frizzled-related protein 1 (REF.[75]). Reportedly, IL-17 from γδ T cells promotes bone formation and facilitates bone fracture healing[76]. It is likely that the effect of IL-17 on bone formation is dependent on the type of osteoblast precursors and the microenvironments of the affected sites[72].

Pro-inflammatory cytokines can modulate osteoblasts by regulating the expression of semaphorins, which are known to act as osteoimmune factors. Under physiological conditions, semaphorin 3A (Sema3A) promotes osteoblastic bone formation, whereas Sema4D suppresses it[77,78]. TNF and IL-6 promote the expression of ADAMTS-4, which cleaves cell-surface Sema4D to generate soluble Sema4D[79]. In RA, serum levels of Sema3A are negatively correlated with disease activity, whereas levels of Sema4D are positively correlated with disease activity, suggesting that altered expression of Sema3A and Sema4D under inflammatory conditions might lead to impaired osteoblastic bone formation in arthritis[79,80].

Whereas immune regulation of osteoblasts has been extensively studied, the regulation of the immune response by osteoblasts in RA remains largely unclear. Within the past few years, it has been reported that osteoblasts produce PLEKHO1, a negative regulator of osteoblastic bone formation that promotes the production of pro-inflammatory cytokines in osteoblasts[81]. Osteoblast-specific inhibition of PLEKHO1 ameliorated inflammation and promoted bone formation in a mouse model of arthritis[81]. Elucidation of osteoblast–immune interactions will contribute to the development of future therapeutic strategies for restoring joint structure.

Periarticular bone loss

Periarticular bone loss in RA is an osteoporotic lesion observed in the bone adjacent to joints[82]. Periarticular bone loss has been attributed to joint inflammation but

the precise mechanism remains unclear. Periarticular bone loss is already present in the pre-RA state in ACPA$^+$ individuals[83]. Indeed, ACPAs were shown to induce osteoclastogenesis and periarticular bone loss in a model of antigen-induced arthritis[84].

Plasma cells are specialized B lineage cells that produce antibodies and reside primarily in the bone marrow[84,85]. Under arthritic conditions, plasma cells accumulate in the bone marrow proximal to inflamed joints and express RANKL at high levels[44]. Plasma cells efficiently induce osteoclastogenesis in vitro in a RANKL-dependent manner[44]. The ability of plasma cells to induce osteoclasts is much greater than that of B cells. Mice deficient in RANKL in the B cell lineage are protected from periarticular bone loss, although not from bone erosion[44]. Plasma cells also produce antibodies and pro-inflammatory cytokines such as IL-6 (REFS[85,86]). Taken together, these findings show that bone marrow plasma cells promote osteoclastogenesis and thereby periarticular bone loss by expressing RANKL, pro-inflammatory cytokines and autoantibodies (FIG. 1). The contribution of RANKL derived from osteoblasts or osteocytes to periarticular bone loss needs to be further explored.

Impaired osteoblastic bone formation results in periarticular bone loss because bone mass that is removed by osteoclasts is not fully replaced. Subchondral bone marrow B cells inhibit osteoblast function by expressing CCL3 and TNF[87]. Expression of sclerostin is increased and expression of RUNX2 is decreased in periarticular bone before the onset of adjuvant-induced arthritis, suggesting that osteocytes may contribute to the impaired bone formation in periarticular bone loss[88].

The bone marrow in proximity to the inflamed joints where periarticular bone loss occurs might be called 'draining' bone marrow, by analogy with draining lymph nodes, which are essential for the initiation and progression of immune responses at inflammatory sites. The immune dysregulation that elicits periarticular bone loss can also trigger joint damage. Patients with RA develop cortical microchannels in the bare area of the joint, where bone is not covered by articular cartilage within the joint capsule at an early stage of the disease, suggesting that the microchannels might facilitate communication between the draining bone marrow and the synovium, leading to the clinical onset and progression of RA[89].

Systemic bone loss

Systemic bone loss in RA is observed as widespread osteoporosis, typically in the vertebrae and femurs, which have an increased risk of fracture compared with those in individuals without RA[8,90,91]. In general, osteoporosis is caused by diverse factors such as ageing, menopause and vitamin D deficiency[11]. The incidence of osteoporosis in patients with RA is approximately two times higher than in the general population[92]. This increased incidence is possibly attributable to RA-specific factors such as activation of the immune system, glucocorticoid treatment and loss of mobility.

Inflammatory factors such as immune complexes and pro-inflammatory cytokines contribute to systemic bone loss, but additional factors, such as glucocorticoid treatment and immobility, are also important. Glucocorticoid treatment inhibits osteoblast differentiation, induces osteoblast apoptosis and activates osteoclast differentiation[90,91]. Pro-inflammatory cytokines, which are produced in the inflamed joints, can induce systemic bone loss by activating osteoclastic bone resorption and inhibiting osteoblastic bone formation in bones at distant sites[90,91]. Immune complexes circulating in the bloodstream stimulate osteoclastic bone resorption systemically[48–50]. As for the source of RANKL in systemic bone loss in RA, plasma cell-derived RANKL is reportedly dispensable, suggesting the importance of RANKL derived from osteoblasts and osteocytes[44]. As mentioned above, a reduction in mechanical loading increases RANKL expression in osteocytes, leading to enhanced osteoclastic bone resorption[10,14]; in addition, mechanical unloading increases sclerostin expression in osteocytes, thereby decreasing osteoblastic bone formation[15]. These mechanisms could explain the immobility-related bone loss in RA (FIG. 1).

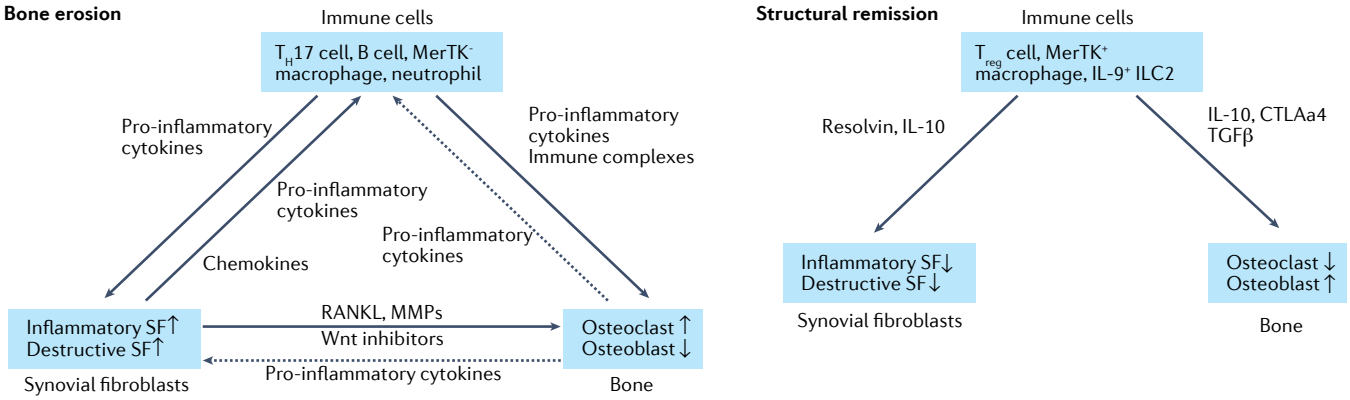
Immune cell–fibroblast–bone interactions

Active disease

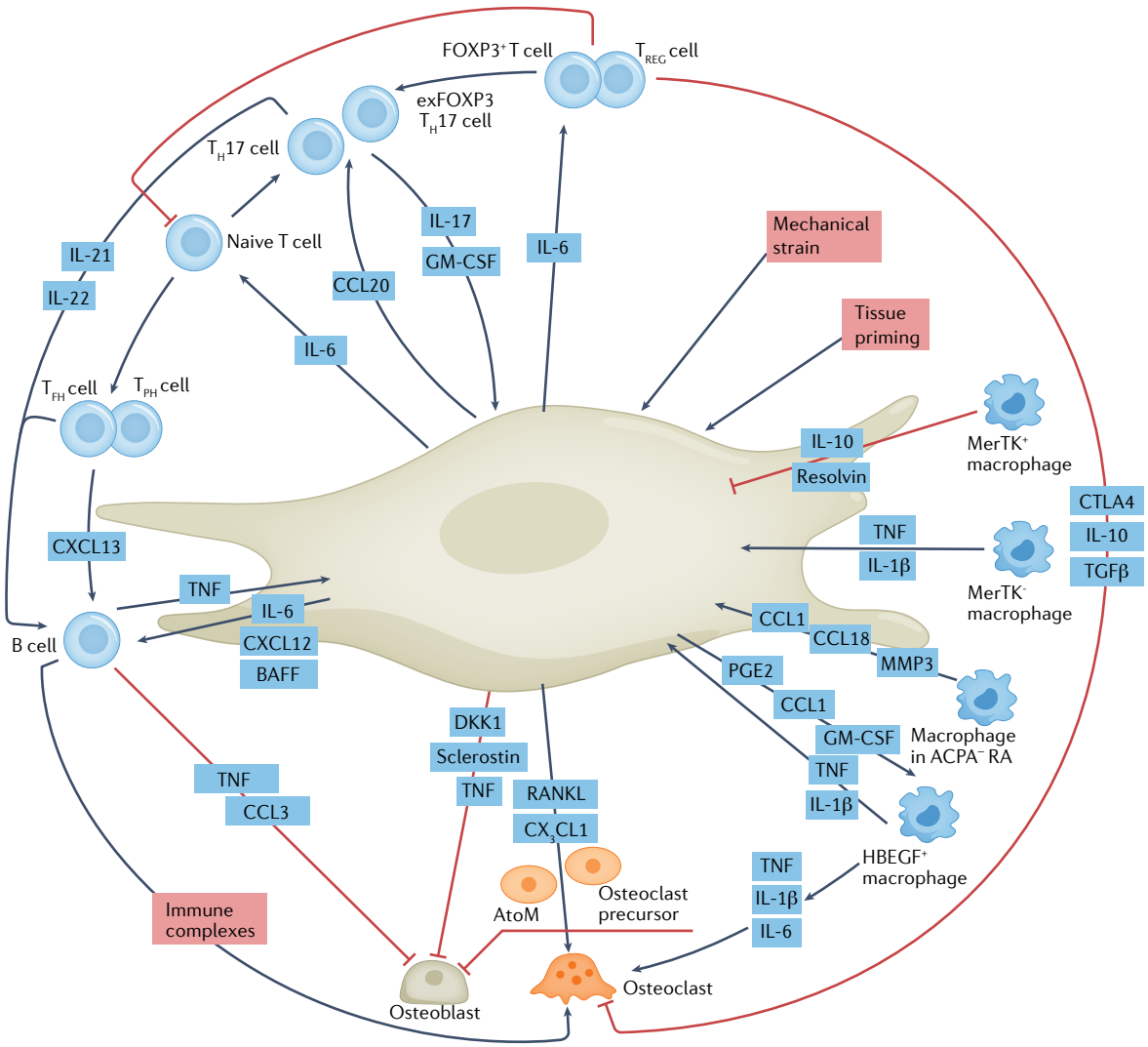
As tissue-destructive synovial fibroblasts are important mediators of joint destruction, it is important to clarify the immune mechanism(s) that promotes the expression of genes encoding tissue-destructive molecules (such as RANKL) in synovial fibroblasts. Early in vitro experiments showed that pro-inflammatory immune mediators, such as IL-17, IL-6, IL-1, TNF, oncostatin-M and prostaglandin E_2, or a combination thereof, increase RANKL expression in synovial fibroblasts[93]. Subsequent studies have provided more information regarding the interactions among immune cells, fibroblasts and bone at the cellular and molecular level.

CD40L on activated T cells was shown in the early studies to induce the proliferation and expression of adhesion molecules, such as intercellular adhesion molecule 1 (ICAM1) and vascular cell adhesion molecule 1 (VCAM1), as well as pro-inflammatory cytokines such as IL-6 by synovial fibroblasts[94]. ICAM1 and VCAM1 expressed by synovial fibroblasts further promote the interaction between T cells and synovial fibroblasts and CD4$^+$ T cell activation[95,96]. T cells and synovial fibroblasts activate each other by producing pro-inflammatory cytokines and chemokines. For instance, T cells induce the expression of pro-inflammatory cytokines such as IL-6 and IL-8 by synovial fibroblasts[97], and IL-7 from synovial fibroblasts promotes a homeostatic proliferation of T cells under arthritic conditions[98]. CX_3CL1 (also known as fractalkine) expressed by synovial fibroblasts promotes the recruitment and activation of CX_3CR1-expressing T cells[99]. Considering the high level of expression of CX_3CR1 on T_{PH} cells, it is possible that T_{PH} cells and synovial fibroblasts might interact with each other[19]. CXCL10 expressed by synovial fibroblasts enhances the recruitment of CXCR3-expressing T cells, including T_H1 cells, and CCL20 from synovial fibroblasts promotes the recruitment of CCR6-expressing T_H17 cells to inflammatory joints[100,101]. Of note, IL-6 produced by synovial fibroblasts promotes the differentiation of T_H17

a Immune cell-fibroblast-bone interplay in rheumatoid arthritis



b



cells, and IL-17 together with IL-6 further enhances IL-6 production by synovial fibroblasts, forming a positive feedback loop referred to as the IL-6 amplifier[102]. IL-6 is also important for the pathogenic conversion of FOXP3⁺ T cells into exFOXP3T_H17 cells. IL-6 produced by synovial fibroblasts determines the fate of plastic FOXP3⁺ T cells and thereby promotes an imbalance between T_{reg} cells and T_H17 cells[62]. In line with this concept, in a clinical study an increase in peripheral blood T_{reg} cells was correlated with clinical response in patients with RA treated with IL-6 blockade[103]. T_H17 cells promote the production of not only RANKL, but also many pro-inflammatory mediators including granulocyte–macrophage colony-stimulating factor (GM-CSF) and

Fig. 2 | **Immune cell–fibroblast–bone interplay in rheumatoid arthritis.** Overview of the interactions among immune cells, fibroblasts and bone in bone erosion and remission. **a** | *Left*: Immune cells and pro-inflammatory synovial fibroblasts interact and activate each other via the production of pro-inflammatory cytokines and chemokines. Tissue-destructive synovial fibroblasts induce osteoclastogenesis by expressing receptor activator of NF-κB ligand (RANKL) and inhibit osteoblastogenesis by expressing Wnt inhibitors. Immune cells can directly promote osteoclastogenesis and inhibit osteoblastogenesis via pro-inflammatory cytokines and immune complexes. Bone cells reportedly activate immune cells and synovial fibroblasts via pro-inflammatory cytokines, but the effects of bone cells on the other cells are not well investigated. *Right*: Immune cell subsets including regulatory T (T_{reg}) cells, MerTK$^+$CD206$^+$ macrophages and IL-9-producing innate lymphoid cells (ILCs) are thought to be involved in structural remission. Regulatory subsets of synovial fibroblasts and bone cells remain to be identified. **b** | Details of the interplay within the immune cell–fibroblast–bone interplay. Integration of findings from single-cell RNA sequencing and biological studies enables us to depict the interactions among immune cells, fibroblasts and bone at the cellular and molecular levels. ACPA, anti-citrullinated peptide antibody; AtoM, arthritis-associated osteoclastogenic macrophage; GM-CSF, granulocyte–macrophage colony-stimulating factor; MMP, matrix metalloproteinase; RA, rheumatoid arthritis; SF, synovial fibroblast; T_{FH} cell, T follicular helper cell; T_H17 cell, T helper 17 cell; T_{PH} cell, T peripheral helper cell.

cadherin-11, by synovial fibroblasts[104,105]. Thus, synovial fibroblasts and T cells activate each other to amplify inflammation and bone erosion in arthritis[7]. RA synovial fibroblasts can reportedly act as antigen-presenting cells by internalizing neutrophil extracellular traps that contain citrullinated peptides and presenting them as antigens to T cells[106,107]. Considering that the proportion of HLA^{hi}THY1$^+$ sublining synovial fibroblasts is increased in the RA synovium[18], it is possible that these synovial fibroblasts may amplify inflammation as antigen-presenting cells.

RA synovial fibroblasts promote the survival of B cells by producing VCAM1 and CXCL12 (REF.[108]). In addition, synovial fibroblasts stimulated with Toll-like receptor 3 ligands promote the differentiation and activation of B cells by producing TNF ligand superfamily members 13 and 13B (also known as APRIL and BAFF, respectively) as well as IL-6, thereby promoting the production of antibodies, including ACPAs[109]. In turn, immune complexes exacerbate inflammation and osteoclastogenesis[3,48–50]. In a study published in 2020, longitudinal genomic analysis of blood from patients with RA revealed that just before a flare of RA the activation of B cells is followed by an expansion of circulating CD3$^-$CD45$^-$PDPN$^+$ pre-inflammatory mesenchymal (PRIME) cells, which resemble pathogenic sublining inflammatory synovial fibroblasts[110]. Levels of PRIME cells then decrease in the blood just after symptom onset and are considered to expand in inflammatory synovium, suggesting that they might migrate from the blood to the synovium. This suggests a possible contribution of an interaction between B cells and synovial fibroblasts to the recurrence of RA symptoms[110]. It would be interesting to clarify where PRIME cells come from and how they are activated, in order to better understand the mechanism of RA flare.

As mentioned above, RA synovial fibroblasts promote the recruitment of monocytes into joints by secreting chemokines such as CCL2 and CXCL10. Mechanical strain-mediated exacerbation of arthritis is dependent on these chemokines, suggesting that the interaction between monocytes and mechanosensitive synovial fibroblasts might underlie the joint specificity of RA pathogenesis[23]. Obviously, the recruitment of RANK$^+$ monocytes and macrophages enhances osteoclastic bone erosion through their interaction with RANKL$^+$ synovial fibroblasts. A study published in 2019 identified a $CX_3CR1^{hi}Ly6C^{int}F4/80^+$I-A/I-E$^+$ macrophage subset, termed arthritis-associated osteoclastogenic macrophages (AtoMs), as the pathogenic osteoclast precursor population in arthritis[111]. Because CX_3CL1 is highly produced by endothelial cells and synovial fibroblasts, the CX_3CR1–CX_3CL1 axis is important for the migration of pathogenic osteoclast precursors to the inflamed synovium. In addition, prostaglandins produced by RA synovial fibroblasts drive the polarization of proheparin-binding EGF-like growth factor (HBEGF)-expressing macrophages[112]; in turn, these HBEGF$^+$ macrophages promote synovial fibroblast invasiveness.

Aside from their interaction with immune cells, synovial fibroblasts interact with mesenchymal cells such as endothelial cells and osteoblasts in arthritis. As mentioned above, the differentiation of inflammatory synovial fibroblasts requires Notch signalling triggered by endothelial cells[30]. Moreover, RA synovial fibroblasts suppress osteoblastic bone formation via the expression of DKK1 (REF.[113]). Although the effects of immune cells on synovial fibroblasts and bone cells have been extensively studied, the influence of bone cells on synovial fibroblasts and/or immune cells has not been fully clarified and needs to be explored in further studies (FIG. 2).

Remission

In patients with clinical remission, joint structural damage typically does not proceed. However, certain patients can be in a state of clinical remission in terms of signs and symptoms of inflammatory joint disease, but can have subclinical synovitis detectable by ultrasonography, which is associated with a high risk of bone erosion[114]. Thus, complete resolution of joint inflammation could be important for the achievement of structural remission with no further bone loss. Conversely, however, treatment with TNF blockade has been reported to suppress joint destruction even in patients who experience no or little clinical improvement, suggesting that joint destruction sometimes proceeds independently of inflammation[115]. Identifying the specific mechanism by which joint destruction occurs would help in the development of a method for establishing structural remission.

As mentioned above, T_{reg} cells have an important role in immune suppression[56]. T_{reg} cells also regulate bone homeostasis by decreasing osteoclastogenesis and increasing osteoblastic bone formation via CTLA4, IL-10 and transforming growth factor-β[61,116–118]. Under physiological conditions, the adoptive transfer of T_{reg} cells suppresses osteoclastogenesis and increases bone volume[119]. Moreover, Foxp3-Tg mice are protected from bone erosion in a model of TNF-Tg arthritis[120]. Impaired T_{reg} cell function or the emergence of exFOXP3T_H17 cells has a pathological role in both autoimmune inflammation and bone resorption[62]. IL-9-deficient mice exhibit

delayed resolution of antigen-induced arthritis as well as impaired activation of T_{reg} cells and impaired proliferation of type 2 innate lymphoid cells (ILC2s)[121]. Administration of IL-9 in a serum transfer-induced arthritis model led to resolution of inflammation and joint destruction. IL-9 induces proliferation of ILC2s, which activate T_{reg} cells in a manner dependent on inducible T cell costimulator (ICOS) and TNF receptor superfamily member 18 (GITR), supporting the importance of the interaction between T_{reg} cells and ILC2s in the resolution phase of arthritis[121]. These findings suggest that controlling T_{reg} cells would be a powerful approach to achieving structural remission.

As for anti-inflammatory subsets of macrophages, a 2019 study identified a population of CX_3CR1^+ resident synovial macrophages that restrict inflammatory reactions by providing a tight-junction-mediated protective barrier for the joint[122]. Determining how CX_3CR1^+ resident synovial macrophages and lining synovial fibroblasts interact with each other under physiological and arthritic conditions would be of interest. Another protective macrophage subset was identified by scRNA-seq analysis of synovial tissue macrophages from patients with early active RA, treatment-refractory active RA or treatment-sensitive RA in remission. These $MerTK^+CD206^+$ macrophages, which are enriched in the synovium of patients with RA in a state of sustained remission, resolve inflammation and induce a 'repair' phenotype of synovial fibroblasts via the production of lipid mediators[123]. Having a low proportion of $MerTK^+$ macrophages is associated with an increased risk of disease flare after treatment cessation. This approach using scRNA-seq analysis will be important for the further identification of regulatory cell subsets that are necessary for inhibiting structural damage in RA (FIG. 2).

Treating structural damage

RA treatment is generally focused on immunomodulatory therapy to address joint inflammation. DMARDs, which are widely used for RA treatment, are now classified into three groups: conventional synthetic DMARDs, such as methotrexate; biologic DMARDs (bDMARDs), including anti-TNF, anti-IL-6 and anti-CD20 agents and CTLA4-Ig; and targeted synthetic DMARDs (tsDMARDs), such as Janus kinase (JAK) inhibitors[8,90].

bDMARDs and JAK inhibitors are effective in preventing both joint inflammation and bone erosion. However, the effects of DMARDs on periarticular and systemic bone loss in RA are limited or have been poorly investigated[90,91]. The anti-RANKL antibody denosumab decreases bone erosion in RA[124–126] and is approved in Japan for the treatment of bone erosion of RA. Anti-RANKL antibodies and bisphosphonates are effective for treating systemic osteoporosis and in reducing the risk of fracture in patients with RA, although they do not exert effects on inflammation or cartilage degradation[126].

Current therapies effectively inhibit the progression of bone destruction in the majority of patients with RA, but in certain cases the response to even multiple DMARDs is inadequate, and it is thus difficult to completely prevent bone destruction. Therefore, we urgently need to fully elucidate the cellular and molecular network underlying structural damage in RA. In this section we provide an overview of the effects of bDMARDs and JAK inhibitors on joint structure and discuss novel candidates for future therapies to treat structural damage.

Biologic DMARDs

TNF inhibitors, IL-6 inhibitors and CTLA4-Ig are widely used bDMARDs. These bDMARDs effectively inhibit inflammation, bone erosion and cartilage degradation by suppressing the local inflammation mediated by synovial fibroblasts and macrophages as well as by inhibiting RANKL induction and RANK signalling pathways[3,8,11]. One might consider that structural protection is achieved mainly by the inhibition of inflammation, but inflammation-independent effects of TNF blockade on bone could exist, given that bone erosion is ameliorated in certain cases without any improvement in inflammation[115]. CTLA4-Ig inhibits inflammation by binding to CD80/CD86 on dendritic cells and suppressing T cell activation, and directly inhibits osteoclast differentiation by inducing apoptosis of osteoclast precursor cells in a CD80/CD86-dependent manner[118,127].

In patients with established RA, inhibitors of IL-17A or IL-23 are less effective than other bDMARDs[128], even though it has been well documented that T_H17 cells are critical to arthritis pathogenesis (both inflammation and bone damage) and IL-17-deficient mice have been shown to be resistant to inflammation and bone destruction in various mouse arthritis models[7]. This reduced efficacy could be attributable to the heterogeneity of RA, with T_H17-dependent mouse models reflecting the disease of only some patients with RA. Alternatively, it is possible that T_H17 cells are important only for the early phase of RA rather than the established phase[20]. In line with this idea, an anti-IL-17 antibody was shown to be effective in the early phase rather than the late phase in a T_H17-dependent mouse model[129]. Notably, dual blockade of IL-17A and IL-17F with bimekizumab produced a favourable result in a clinical trial involving patients with RA who had an inadequate response to a TNF inhibitor, suggesting that IL-17 blockade remains a promising approach if IL-17 family cytokines are fully blocked[130]. The current therapies for RA target pro-inflammatory cytokines mainly produced by innate immune cells and synovial fibroblasts, and thus target bystander pathways rather than antigen-specific pathways. Understanding the autoimmune mechanisms in RA could lead to the establishment of new therapeutic strategies in the future.

JAK inhibitors

JAKs (including JAK1, JAK2, JAK3 and TYK2) are widely expressed in immune and stromal cells in joints and are involved in various cellular responses initiated by cytokines. JAKs phosphorylate signal transducer and activator of transcription proteins (STATs), which then translocate to the nucleus to regulate gene transcription. JAK inhibitors suppress joint inflammation to an extent similar to the suppression produced by bDMARDs[8,90]. Which type of cells and signalling

pathways are the specific targets of JAK inhibitors in vivo remains unclear, as most immune and bone cells are influenced by cytokine signalling that utilizes JAK–STAT pathways. In vitro studies have shown that JAK inhibitors suppress the production of IFNγ and IL-17 as well as the proliferation of $CD4^+$ T cells[131]. JAK inhibitors also inhibit the expression of CD80/CD86 as well as pro-inflammatory cytokines such as IL-6 and TNF in dendritic cells[132].

Certain JAK inhibitors inhibit bone erosion in patients with RA more potently than TNF blockade, suggesting that some JAK inhibitors might protect against structural damage through distinct mechanisms[133,134]. Although JAK inhibitors have no direct effects on osteoclast precursors, they suppress osteoclastogenesis by inhibiting the expression of RANKL on osteoclast-supporting mesenchymal cells[135,136]. In vitro, JAK inhibitors promote osteoblastogenesis in part by increasing the expression of anabolic proteins such as Wnt1 and β-catenin in osteoblasts[135]. In addition, it seems that JAK inhibition reverses bone erosion in RA by promoting the restoration of bone mass[135]. Further studies are necessary to elucidate whether and how JAK inhibition regulates structural damage in vivo.

Emerging therapeutic targets

To reinstate the joint structure, it is necessary to determine how to enhance the osteoblastic bone formation under arthritic conditions[137]. Blockade of DKK1 and sclerostin, both of which inhibit Wnt signalling, have been shown to exert considerable effects on bone formation in arthritis[67,113,138,139]. Treatment with an anti-DKK1 antibody prevents bone damage and leads to bone formation in TNF-Tg arthritis[113]. An anti-sclerostin antibody blocks periarticular and systemic bone loss in TNF-Tg arthritis and CIA, although it does not affect joint inflammation[138,139]. Thus, blockade with Wnt inhibitors could serve as a treatment for reinstating the joint structure in RA. However, TNF-Tg arthritis is exacerbated in sclerostin-deficient mice, consistent with a role for sclerostin in attenuating TNF signalling[67]. Therefore, treatment with a sclerostin inhibitor needs to be carefully conducted with much attention given to potential adverse effects.

There are other candidate molecules that can increase bone formation under arthritic conditions. Sema3A exerts bone anabolic effects by increasing osteoblastic formation and inhibiting osteoclastogenesis[77]. Sema3A has also been identified as an immunosuppressive factor, and the administration of Sema3A not only ameliorated inflammation and bone erosion but also increased bone formation in a serum transfer-induced model of arthritis[140]. In addition, Sema4D is known to be an osteoimmune molecule that promotes inflammation and inhibits osteoblastic bone formation[78,79]. Administration of an anti-Sema4D antibody inhibits inflammation and bone erosion in CIA[79]. Moreover, Notch signalling is important for the polarization of inflammatory synovial fibroblasts as well as the inhibition of osteoblastic bone formation, and studies in mice have shown that Notch inhibition increases bone volume by enhancing osteoblastic bone formation[141]. Furthermore, the CX_3CR1–CX_3CL1 axis and the CXCL10–CXCR3 axis promote not only the migration of T cells and macrophages, but also the activation of synovial fibroblasts[99,100,142,143]. Blockade of CX_3CL1 and CXCL10 as well as blockade of GM-CSF and M-CSF have been shown to inhibit inflammation and bone erosion in mouse models of arthritis and are now being investigated in a clinical trial[100,144–148]. Thus, therapeutic strategies targeting molecules involved in the immune cell–fibroblast–bone triad will be beneficial for both inhibition of inflammation and restoration of the joint structure in RA (TABLE 1).

Current therapies are not universally effective in all patients because RA pathogenesis is heterogeneous. The lack of predictors of treatment success presents a problem in relation to the choice of the best therapy for each individual patient. It is thus important to establish therapeutic strategies that are based on patient subpopulations. It remains to be seen whether the analysis of cells and transcriptomes in synovial tissue samples can appropriately delineate disease subsets and provide better targets for therapeutics. Alternatively, targeting the pathogenic synovial fibroblasts common to all patients with RA is an attractive therapeutic strategy. In terms of targeting surface molecules expressed on synovial fibroblasts, administration of antibodies directed against cadherin-11 and depletion of $FAP\alpha^+$ synovial fibroblasts have been shown to be effective in mouse models of RA[4,28]. An anti-cadherin-11 antibody has been shown to be ineffective in clinical studies, while therapies targeting FAPα are still under clinical investigation[149,150]. At present, there are no therapies targeting synovial fibroblasts that inhibit both bone erosion and cartilage degradation. Further identification of the surface or intracellular proteins specifically expressed by tissue-destructive synovial fibroblasts will contribute to the development of agents designed to treat structural damage.

Conclusions

Synovial fibroblasts play an important part in exacerbating inflammation and joint damage in RA by enhancing osteoclastogenic bone erosion and cartilage destruction as well as inhibiting osteoblastic bone formation. Structural remission will be achieved by completely inhibiting inflammation in addition to inhibiting the specific pathways related to joint damage. To this end, it will be important to further elucidate the mechanisms of immune cell–fibroblast–bone interplay and their effects on joint destruction and the generation of pathogenic synovial fibroblasts. T_H17 cells has been considered to play a key role in autoimmune inflammation and bone destruction[3,12]. The activation of immune cells, including induction of a T_{reg} cell–T_H17 cell imbalance, is important for the arthritogenic effects of synovial fibroblasts. T_{reg} cells not only inhibit inflammation, but also inhibit osteoclastogenic bone resorption and promote osteoblastic bone formation. It would be interesting to investigate whether T_{reg} cells modulate joint damage by regulating the function or polarization of synovial fibroblasts. Thus, in future studies more attention will need to paid to T_{reg} cells and synovial fibroblasts as well as cells that promote bone formation.

Table 1 | **The effect of molecules on immune cells, synovial fibroblasts and bone**

Molecule	Main source	Effect on immune cells	Effect on synovial fibroblasts	Effect on bone
Established therapeutic targets for treatment of RA				
TNF	Macrophages, T cells, B cells	Activation	Activation, ↑ RANKL expression	Osteoblasts ↓, osteoclasts ↑
IL-6	Synovial fibroblasts	Activation, T_{reg} cell–T_H17 cell imbalance	Activation, ↑ RANKL expression	Osteoblasts ↓, osteoclasts ↑
IL-1β	Macrophages	Activation	Activation, ↑ RANKL expression	Osteoblasts ↓, osteoclasts ↑
CTLA4	T_{reg} cells	Inhibition of T cell priming and DCs	ND	Osteoclasts ↓, osteoblasts ↑[a]
IL-17	T_H17 cells	Activation, accumulation	Activation, ↑ RANKL expression	Osteoblasts ↓, osteoclasts ↑[b]
JAKs	Various cell types	Activation	Activation, ↑ RANKL expression	Osteoblasts ↓, osteoclasts ↑[b]
Autoantibodies	B cells	Activation	ND	Osteoclasts ↑
Selected candidate therapeutic targets for treatment of RA				
Sema3A	Osteoblasts, synovial fibroblasts	Inhibition	Activation	Osteoblasts ↑, osteoclasts ↓
Sema4D	T cells	Activation	ND	Osteoblasts ↓
NOTCH3	Synovial fibroblasts	Activation	Polarization of pro-inflammatory synovial fibroblasts	Osteoblasts ↓
Cadherin-11	Synovial fibroblasts	ND	Activation, cell–cell adhesion for the maintenance of synovial architecture	ND
DKK1	Synovial fibroblasts	ND	ND	Osteoblasts ↓
Sclerostin	Osteocytes, synovial fibroblasts	ND	ND	Osteoblasts ↓
CXCL10	Synovial fibroblasts	Recruitment of T cells	Activation	ND
Fractalkine (CX_3CL1)	Synovial fibroblasts, endothelial cells	Recruitment of T cells and monocytes	Activation	Osteoclasts ↑
GM-CSF	Synovial fibroblasts, T cells, ILCs	Activation of DCs and macrophages	ND	Osteoclasts ↓
M-CSF	Mesenchymal cells	Activation of macrophages	ND	Osteoclasts ↑

DC, dendritic cell; GM-CSF, granulocyte–macrophage colony-stimulating factor; ILC, innate lymphoid cell; JAK, Janus kinase; M-CSF, macrophage colony-stimulating factor; ND, not determined; RANKL, receptor activator of NF-κB ligand; T_H17 cell, T helper 17 cell; T_{reg} cell, regulatory T cell. [a]CTLA4 indirectly promotes osteoblastic bone formation by inducing T cell anergy and production of T cell derived Wnt10b. [b]IL-17 and JAK indirectly promote osteoclastic bone absorption by inducing RANKL expression on synovial fibroblasts.

Technological advances in the past several years have revealed the heterogeneity of cell subsets and enabled the identification of pathogenic and protective cell populations. From a therapeutic point of view, it is important to restore the joint structure by increasing osteoblastic bone formation and by targeting the pathogenic immune cell–fibroblast axis. Clarification of the interaction between immune cells and fibroblasts at the single-cell level will provide new insights into the pathogenesis of RA. In order to prove the pathological relevance of the findings obtained by scRNA-seq analysis, it will be necessary to perform loss-of-function analysis in vivo, such as cell-type-specific gene deletion. Clarifying how skeletal stem cells or the nervous system contribute to joint damage in RA will also be important. Integration of *in silico* and in vivo studies will provide a complete atlas of the immune cell–fibroblast–bone triad in RA, providing a molecular basis for the development of future therapeutic strategies aimed at providing protection against structural damage as well as restoration of damaged joints.

Published online 15 June 2022

1. Firestein, G. S. Evolving concepts of rheumatoid arthritis. *Nature* **423**, 356–361 (2003).
2. McInnes, I. B. & Schett, G. The pathogenesis of rheumatoid arthritis. *N. Engl. J. Med.* **365**, 2205–2219 (2011).
3. Takayanagi, H. Osteoimmunology: shared mechanisms and crosstalk between the immune and bone systems. *Nat. Rev. Immunol.* **7**, 292–304 (2007).
4. Croft, A. P. et al. Distinct fibroblast subsets drive inflammation and damage in arthritis. *Nature* **570**, 246–251 (2019).
5. Mizoguchi, F. et al. Functionally distinct disease-associated fibroblast subsets in rheumatoid arthritis. *Nat. Commun.* **9**, 789 (2018).
6. Danks, L. et al. RANKL expressed on synovial fibroblasts is primarily responsible for bone erosions during joint inflammation. *Ann. Rheum. Dis.* **75**, 1187–1195 (2016).
7. Komatsu, N. & Takayanagi, H. Inflammation and bone destruction in arthritis: synergistic activity of immune and mesenchymal cells in joints. *Front. Immunol.* **3**, 77 (2012).
8. Shim, J. H., Stavre, Z. & Gravallese, E. M. Bone loss in rheumatoid arthritis: basic mechanisms

and clinical implications. *Calcif. Tissue Int.* **102**, 533–546 (2018).
9. Nakashima, T. et al. Evidence for osteocyte regulation of bone homeostasis through RANKL expression. *Nat. Med.* **17**, 1231–1234 (2011).
10. Xiong, J. et al. Matrix-embedded cells control osteoclast formation. *Nat. Med.* **17**, 1235–1241 (2011).
11. Okamoto, K. et al. Osteoimmunology: the conceptual framework unifying the immune and skeletal systems. *Physiol. Rev.* **97**, 1295–1349 (2017).
12. Takayanagi, H. et al. Induction and activation of the transcription factor NFATc1 (NFAT2) integrate RANKL signaling in terminal differentiation of osteoclasts. *Dev. Cell* **3**, 889–901 (2002).
13. Tsukasaki, M. et al. OPG production matters where it happened. *Cell Rep.* **32**, 108124 (2020).
14. Robling, A. G. et al. Mechanical stimulation of bone in vivo reduces osteocyte expression of Sost/sclerostin. *J. Biol. Chem.* **283**, 5866–5875 (2008).
15. Miyazaki, T. et al. Mechanical regulation of bone homeostasis through p130Cas-mediated alleviation of NF-κB activity. *Sci. Adv.* **5**, eaau7802 (2019).
16. Davidson, S. et al. Fibroblasts as immune regulators in infection, inflammation and cancer. *Nat. Rev. Immunol.* **21**, 704–717 (2021).
17. Nygaard, G. & Firestein, G. S. Restoring synovial homeostasis in rheumatoid arthritis by targeting fibroblast-like synoviocytes. *Nat. Rev. Rheumatol.* **16**, 316–333 (2020).
18. Zhang, F. et al. Defining inflammatory cell states in rheumatoid arthritis joint synovial tissues by integrating single-cell transcriptomics and mass cytometry. *Nat. Immunol.* **20**, 928–942 (2019).
19. Rao, D. A. et al. Pathologically expanded peripheral T helper cell subset drives B cells in rheumatoid arthritis. *Nature* **542**, 110–114 (2017).
20. Pfeifle, R. et al. Regulation of autoantibody activity by the IL-23–T_H17 axis determines the onset of autoimmune disease. *Nat. Immunol.* **18**, 104–113 (2017).
21. Bondt, A. et al. ACPA IgG galactosylation associates with disease activity in pregnant patients with rheumatoid arthritis. *Ann. Rheum. Dis.* **77**, 1130–1136 (2018).
22. Chang, M. H. et al. Arthritis flares mediated by tissue-resident memory T cells in the joint. *Cell Rep.* **37**, 109902 (2021).
23. Cambre, I. et al. Mechanical strain determines the site-specific localization of inflammation and tissue damage in arthritis. *Nat. Commun.* **9**, 4613 (2018).
24. Wu, X. et al. Single-cell sequencing of immune cells from anticitrullinated peptide antibody positive and negative rheumatoid arthritis. *Nat. Commun.* **12**, 4977 (2021).
25. Karouzakis, E. et al. Analysis of early changes in DNA methylation in synovial fibroblasts of RA patients before diagnosis. *Sci. Rep.* **8**, 7370 (2018).
26. Hua, S. & Dias, T. H. Hypoxia-inducible factor (HIF) as a target for novel therapies in rheumatoid arthritis. *Front. Pharmacol.* **7**, 184 (2016).
27. Friscic, J. et al. The complement system drives local inflammatory tissue priming by metabolic reprogramming of synovial fibroblasts. *Immunity* **54**, 1002–1021 (2021).
28. Lee, D. M. et al. Cadherin-11 in synovial lining formation and pathology in arthritis. *Science* **315**, 1006–1010 (2007).
29. Chang, S. K. et al. Cadherin-11 regulates fibroblast inflammation. *Proc. Natl Acad. Sci. USA* **108**, 8402–8407 (2011).
30. Wei, K. et al. Notch signalling drives synovial fibroblast identity and arthritis pathology. *Nature* **582**, 259–264 (2020).
31. Armaka, M. et al. Mesenchymal cell targeting by TNF as a common pathogenic principle in chronic inflammatory joint and intestinal diseases. *J. Exp. Med.* **205**, 331–337 (2008).
32. Armaka, M., Ospelt, C., Pasparakis, M. & Kollias, G. The p55TNFR-IKK2-Ripk3 axis orchestrates arthritis by regulating death and inflammatory pathways in synovial fibroblasts. *Nat. Commun.* **9**, 618 (2018).
33. Nguyen, H. N. et al. Autocrine loop involving IL-6 family member LIF, LIF receptor, and STAT4 drives sustained fibroblast production of inflammatory mediators. *Immunity* **46**, 220–232 (2017).
34. Bromley, M. & Woolley, D. E. Chondroclasts and osteoclasts at subchondral sites of erosion in the rheumatoid joint. *Arthritis Rheum.* **27**, 968–975 (1984).
35. Takayanagi, H. et al. Involvement of receptor activator of nuclear factor κB ligand/osteoclast differentiation factor in osteoclastogenesis from synoviocytes in rheumatoid arthritis. *Arthritis Rheum.* **43**, 259–269 (2000).
36. Takayanagi, H. et al. A new mechanism of bone destruction in rheumatoid arthritis: synovial fibroblasts induce osteoclastogenesis. *Biochem. Biophys. Res. Commun.* **240**, 279–286 (1997).
37. Gravallese, E. M. et al. Synovial tissue in rheumatoid arthritis is a source of osteoclast differentiation factor. *Arthritis Rheum.* **43**, 250–258 (2000).
38. Pettit, A. R. et al. TRANCE/RANKL knockout mice are protected from bone erosion in a serum transfer model of arthritis. *Am. J. Pathol.* **159**, 1689–1699 (2001).
39. Redlich, K. et al. Osteoclasts are essential for TNF-α-mediated joint destruction. *J. Clin. Invest.* **110**, 1419–1427 (2002).
40. Takayanagi, H. et al. T-cell-mediated regulation of osteoclastogenesis by signalling cross-talk between RANKL and IFN-γ. *Nature* **408**, 600–605 (2000).
41. Kong, Y. Y. et al. Activated T cells regulate bone loss and joint destruction in adjuvant arthritis through osteoprotegerin ligand. *Nature* **402**, 304–309 (1999).
42. Meednu, N. et al. Production of RANKL by memory B cells: a link between B cells and bone erosion in rheumatoid arthritis. *Arthritis Rheumatol.* **68**, 805–816 (2016).
43. Ota, Y. et al. Generation mechanism of RANKL$^+$ effector memory B cells: relevance to the pathogenesis of rheumatoid arthritis. *Arthritis Res. Ther.* **18**, 67 (2016).
44. Komatsu, N. et al. Plasma cells promote osteoclastogenesis and periarticular bone loss in autoimmune arthritis. *J. Clin. Invest.* **131**, e143060 (2021).
45. Herman, S. et al. Induction of osteoclast-associated receptor, a key osteoclast costimulation molecule, in rheumatoid arthritis. *Arthritis Rheum.* **58**, 3041–3050 (2008).
46. Lam, J. et al. TNF-α induces osteoclastogenesis by direct stimulation of macrophages exposed to permissive levels of RANK ligand. *J. Clin. Invest.* **106**, 1481–1488 (2000).
47. Ochi, S. et al. Pathological role of osteoclast costimulation in arthritis-induced bone loss. *Proc. Natl Acad. Sci. USA* **104**, 11394–11399 (2007).
48. Negishi-Koga, T. et al. Immune complexes regulate bone metabolism through FcRγ signalling. *Nat. Commun.* **6**, 6637 (2015).
49. Krishnamurthy, A. et al. Identification of a novel chemokine-dependent molecular mechanism underlying rheumatoid arthritis-associated autoantibody-mediated bone loss. *Ann. Rheum. Dis.* **75**, 721–729 (2016).
50. Harre, U. et al. Induction of osteoclastogenesis and bone loss by human autoantibodies against citrullinated vimentin. *J. Clin. Invest.* **122**, 1791–1802 (2012).
51. Burska, A. N. et al. Receptor activator of nuclear factor κ-B ligand (RANKL) serum levels are associated with progression to seropositive/negative rheumatoid arthritis. *Clin. Exp. Rheumatol.* **39**, 456–462 (2021).
52. Asano, T. et al. Soluble RANKL is physiologically dispensable but accelerates tumour metastasis to bone. *Nat. Metab.* **1**, 868–875 (2019).
53. Sato, K. et al. T_H17 functions as an osteoclastogenic helper T cell subset that links T cell activation and bone destruction. *J. Exp. Med.* **203**, 2673–2682 (2006).
54. Kotake, S. et al. IL-17 in synovial fluids from patients with rheumatoid arthritis is a potent stimulator of osteoclastogenesis. *J. Clin. Invest.* **103**, 1345–1352 (1999).
55. Harre, U. et al. Glycosylation of immunoglobulin G determines osteoclast differentiation and bone loss. *Nat. Commun.* **6**, 6651 (2015).
56. Sakaguchi, S., Yamaguchi, T., Nomura, T. & Ono, M. Regulatory T cells and immune tolerance. *Cell* **133**, 775–787 (2008).
57. Hori, S., Nomura, T. & Sakaguchi, S. Control of regulatory T cell development by the transcription factor Foxp3. *Science* **299**, 1057–1061 (2003).
58. Okada, Y. et al. Genetics of rheumatoid arthritis contributes to biology and drug discovery. *Nature* **506**, 376–381 (2014).
59. Ota, M. et al. Dynamic landscape of immune cell-specific gene regulation in immune-mediated diseases. *Cell* **184**, 3006–3021 (2021).
60. Zaiss, M. M. et al. T_{reg} cells suppress osteoclast formation: a new link between the immune system and bone. *Arthritis Rheum.* **56**, 4104–4112 (2007).
61. Komatsu, N. & Takayanagi, H. Regulatory T cells in arthritis. *Prog. Mol. Biol. Transl. Sci.* **136**, 207–215 (2015).
62. Komatsu, N. et al. Pathogenic conversion of FOXP3$^+$ T cells into T_H17 cells in autoimmune arthritis. *Nat. Med.* **20**, 62–68 (2014).
63. Kochi, Y. et al. A regulatory variant in CCR6 is associated with rheumatoid arthritis susceptibility. *Nat. Genet.* **42**, 515–519 (2010).
64. Walsh, N. C. et al. Osteoblast function is compromised at sites of focal bone erosion in inflammatory arthritis. *J. Bone Miner. Res.* **24**, 1572–1585 (2009).
65. Gilbert, L. et al. Expression of the osteoblast differentiation factor RUNX2 (Cbfa1/AML3/Pebp2αA) is inhibited by tumor necrosis factor-α. *J. Biol. Chem.* **277**, 2695–2701 (2002).
66. de Rooy, D. P. et al. Genetic studies on components of the Wnt signalling pathway and the severity of joint destruction in rheumatoid arthritis. *Ann. Rheum. Dis.* **72**, 769–775 (2013).
67. Wehmeyer, C. et al. Sclerostin inhibition promotes TNF-dependent inflammatory joint destruction. *Sci. Transl. Med.* **8**, 330ra335 (2016).
68. Matzelle, M. M. et al. Inflammation in arthritis induces expression of BMP3, an inhibitor of bone formation. *Scand. J. Rheumatol.* **45**, 379–383 (2016).
69. Stashenko, P., Dewhirst, F. E., Rooney, M. L., Desjardins, L. A. & Heeley, J. D. Interleukin-1β is a potent inhibitor of bone formation in vitro. *J. Bone Miner. Res.* **2**, 559–565 (1987).
70. Bellido, T., Borba, V. Z., Roberson, P. & Manolagas, S. C. Activation of the Janus kinase/STAT (signal transducer and activator of transcription) signal transduction pathway by interleukin-6-type cytokines promotes osteoblast differentiation. *Endocrinology* **138**, 3666–3676 (1997).
71. McGregor, N. E. et al. IL-6 exhibits both cis- and trans-signaling in osteocytes and osteoblasts, but only trans-signaling promotes bone formation and osteoclastogenesis. *J. Biol. Chem.* **294**, 7850–7863 (2019).
72. Gravallese, E. M. & Schett, G. Effects of the IL-23–IL-17 pathway on bone in spondyloarthritis. *Nat. Rev. Rheumatol.* **14**, 631–640 (2018).
73. Kampylafka, E. et al. Resolution of synovitis and arrest of catabolic and anabolic bone changes in patients with psoriatic arthritis by IL-17A blockade with secukinumab: results from the prospective PSARTROS study. *Arthritis Res. Ther.* **20**, 153 (2018).
74. Sherlock, J. P. et al. IL-23 induces spondyloarthropathy by acting on ROR-γt$^+$ CD3$^+$CD4$^-$CD8$^-$ entheseal resident T cells. *Nat. Med.* **18**, 1069–1076 (2016).
75. Shaw, A. T., Maeda, Y. & Gravallese, E. M. IL-17A deficiency promotes periosteal bone formation in a model of inflammatory arthritis. *Arthritis Res. Ther.* **18**, 104 (2016).
76. Ono, T. et al. IL-17-producing γδ T cells enhance bone regeneration. *Nat. Commun.* **7**, 10928 (2016).
77. Hayashi, M. et al. Osteoprotection by semaphorin 3A. *Nature* **485**, 69–74 (2012).
78. Negishi-Koga, T. et al. Suppression of bone formation by osteoclastic expression of semaphorin 4D. *Nat. Med.* **17**, 1473–1480 (2011).
79. Yoshida, Y. et al. Semaphorin 4D contributes to rheumatoid arthritis by inducing inflammatory cytokine production: pathogenic and therapeutic implications. *Arthritis Rheumatol.* **67**, 1481–1490 (2015).
80. Takagawa, S. et al. Decreased semaphorin3A expression correlates with disease activity and histological features of rheumatoid arthritis. *BMC Musculoskelet. Disord.* **14**, 40 (2013).
81. He, X. et al. Osteoblastic PLEKHO1 contributes to joint inflammation in rheumatoid arthritis. *EBioMedicine* **41**, 538–555 (2019).
82. Goldring, S. R. Periarticular bone changes in rheumatoid arthritis: pathophysiological implications and clinical utility. *Ann. Rheum. Dis.* **68**, 297–299 (2009).
83. Kleyer, A. et al. Bone loss before the clinical onset of rheumatoid arthritis in subjects with anticitrullinated protein antibodies. *Ann. Rheum. Dis.* **73**, 854–860 (2014).

84. Engdahl, C. et al. Periarticular bone loss in arthritis is induced by autoantibodies against citrullinated vimentin. *J. Bone Miner. Res.* **32**, 1681–1691 (2017).
85. Lightman, S. M., Utley, A. & Lee, K. P. Survival of long-lived plasma cells (LLPC): piecing together the puzzle. *Front. Immunol.* **10**, 965 (2019).
86. Pioli, P. D. Plasma cells, the next generation: beyond antibody secretion. *Front. Immunol.* **10**, 2768 (2019).
87. Sun, W. et al. B cells inhibit bone formation in rheumatoid arthritis by suppressing osteoblast differentiation. *Nat. Commun.* **9**, 5127 (2018).
88. Courbon, G. et al. Early sclerostin expression explains bone formation inhibition before arthritis onset in the rat adjuvant-induced arthritis model. *Sci. Rep.* **8**, 3492 (2018).
89. Werner, D. et al. Early changes of the cortical micro-channel system in the bare area of the joints of patients with rheumatoid arthritis. *Arthritis Rheumatol.* **69**, 1580–1587 (2017).
90. Tanaka, Y. Managing osteoporosis and joint damage in patients with rheumatoid arthritis: an overview. *J. Clin. Med.* **10**, 1241 (2021).
91. Dubrovsky, A. M., Lim, M. J. & Lane, N. E. Osteoporosis in rheumatic diseases: anti-rheumatic drugs and the skeleton. *Calcif. Tissue Int.* **102**, 607–618 (2018).
92. Haugeberg, G., Uhlig, T., Falch, J. A., Halse, J. I. & Kvien, T. K. Bone mineral density and frequency of osteoporosis in female patients with rheumatoid arthritis: results from 394 patients in the Oslo County Rheumatoid Arthritis register. *Arthritis Rheum.* **43**, 522–530 (2000).
93. Kim, K. W., Kim, H. R., Kim, B. M., Cho, M. L. & Lee, S. H. T_H17 cytokines regulate osteoclastogenesis in rheumatoid arthritis. *Am. J. Pathol.* **185**, 3011–3024 (2015).
94. Yellin, M. J. et al. Ligation of CD40 on fibroblasts induces CD54 (ICAM-1) and CD106 (VCAM-1) up-regulation and IL-6 production and proliferation. *J. Leukoc. Biol.* **58**, 209–216 (1995).
95. Van Seventer, G. A., Shimizu, Y., Horgan, K. J. & Shaw, S. The LFA-1 ligand ICAM-1 provides an important costimulatory signal for T cell receptor-mediated activation of resting T cells. *J. Immunol.* **144**, 4579–4586 (1990).
96. Damle, N. K. & Aruffo, A. Vascular cell adhesion molecule 1 induces T-cell antigen receptor-dependent activation of $CD4^+$ T lymphocytes. *Proc. Natl Acad. Sci. USA* **88**, 6403–6407 (1991).
97. Yamamura, Y. et al. Effector function of resting T cells: activation of synovial fibroblasts. *J. Immunol.* **166**, 2270–2275 (2001).
98. Sawa, S. et al. Autoimmune arthritis associated with mutated interleukin (IL)-6 receptor gp130 is driven by STAT3/IL-7-dependent homeostatic proliferation of $CD4^+$ T cells. *J. Exp. Med.* **203**, 1459–1470 (2006).
99. Sawai, H. et al. T cell costimulation by fractalkine-expressing synoviocytes in rheumatoid arthritis. *Arthritis Rheum.* **52**, 1392–1401 (2005).
100. Lee, J. H. et al. Pathogenic roles of CXCL10 signaling through CXCR3 and TLR4 in macrophages and T cells: relevance for arthritis. *Arthritis Res. Ther.* **19**, 163 (2017).
101. Hirota, K. et al. Preferential recruitment of CCR6-expressing T_H17 cells to inflamed joints via CCL20 in rheumatoid arthritis and its animal model. *J. Exp. Med.* **204**, 2803–2812 (2007).
102. Ogura, H. et al. Interleukin-17 promotes autoimmunity by triggering a positive-feedback loop via interleukin-6 induction. *Immunity* **29**, 628–636 (2008).
103. Kikuchi, J. et al. Peripheral blood $CD4^+CD25^+CD127^{low}$ regulatory T cells are significantly increased by tocilizumab treatment in patients with rheumatoid arthritis: increase in regulatory T cells correlates with clinical response. *Arthritis Res. Ther.* **17**, 10 (2015).
104. Hirota, K. et al. Autoimmune T_H17 cells induced synovial stromal and innate lymphoid cell secretion of the cytokine GM-CSF to initiate and augment autoimmune arthritis. *Immunity* **48**, 1220–1232 (2018).
105. Park, Y. E. et al. IL-17 increases cadherin-11 expression in a model of autoimmune experimental arthritis and in rheumatoid arthritis. *Immunol. Lett.* **140**, 97–103 (2011).
106. Tran, C. N. et al. Presentation of arthritogenic peptide to antigen-specific T cells by fibroblast-like synoviocytes. *Arthritis Rheum.* **56**, 1497–1506 (2007).
107. Carmona-Rivera, C. et al. Synovial fibroblast–neutrophil interactions promote pathogenic adaptive immunity in rheumatoid arthritis. *Sci. Immunol.* **2**, eaag3358 (2017).
108. Burger, J. A., Zvaifler, N. J., Tsukada, N., Firestein, G. S. & Kipps, T. J. Fibroblast-like synoviocytes support B-cell pseudoemperipolesis via a stromal cell-derived factor-1- and CD106 (VCAM-1)-dependent mechanism. *J. Clin. Invest.* **107**, 305–315 (2001).
109. Bombardieri, M. et al. A BAFF/APRIL-dependent TLR3-stimulated pathway enhances the capacity of rheumatoid synovial fibroblasts to induce AID expression and Ig class-switching in B cells. *Ann. Rheum. Dis.* **70**, 1857–1865 (2011).
110. Orange, D. E. et al. RNA identification of PRIME cells predicting rheumatoid arthritis flares. *N. Engl. J. Med.* **383**, 218–228 (2020).
111. Hasegawa, T. et al. Identification of a novel arthritis-associated osteoclast precursor macrophage regulated by FoxM1. *Nat. Immunol.* **20**, 1631–1643 (2019).
112. Kuo, D. et al. $HBEGF^+$ macrophages in rheumatoid arthritis induce fibroblast invasiveness. *Sci. Transl. Med.* **11**, eaau8587 (2019).
113. Diarra, D. et al. Dickkopf-1 is a master regulator of joint remodeling. *Nat. Med.* **13**, 156–163 (2007).
114. Brown, A. K. et al. An explanation for the apparent dissociation between clinical remission and continued structural deterioration in rheumatoid arthritis. *Arthritis Rheum.* **58**, 2958–2967 (2008).
115. Smolen, J. S. et al. Evidence of radiographic benefit of treatment with infliximab plus methotrexate in rheumatoid arthritis patients who had no clinical improvement: a detailed subanalysis of data from the Anti-Tumor Necrosis Factor Trial in Rheumatoid Arthritis with Concomitant Therapy study. *Arthritis Rheum.* **52**, 1020–1030 (2005).
116. Roser-Page, S., Vikulina, T., Zayzafoon, M. & Weitzmann, M. N. CTLA-4Ig-induced T cell anergy promotes Wnt-10b production and bone formation in a mouse model. *Arthritis Rheumatol.* **66**, 990–999 (2014).
117. Tyagi, A. M. et al. The microbial metabolite butyrate stimulates bone formation via T regulatory cell-mediated regulation of WNT10B expression. *Immunity* **49**, 1116–1131 (2018).
118. Bozec, A. et al. T cell costimulation molecules CD80/86 inhibit osteoclast differentiation by inducing the IDO/tryptophan pathway. *Sci. Transl. Med.* **6**, 235ra60 (2014).
119. Zaiss, M. M. et al. Increased bone density and resistance to ovariectomy-induced bone loss in FoxP3-transgenic mice based on impaired osteoclast differentiation. *Arthritis Rheum.* **62**, 2328–2338 (2010).
120. Zaiss, M. M. et al. Regulatory T cells protect from local and systemic bone destruction in arthritis. *J. Immunol.* **184**, 7238–7246 (2010).
121. Rauber, S. et al. Resolution of inflammation by interleukin-9-producing type 2 innate lymphoid cells. *Nat. Med.* **23**, 938–944 (2017).
122. Culemann, S. et al. Locally renewing resident synovial macrophages provide a protective barrier for the joint. *Nature* **572**, 670–675 (2019).
123. Alivernini, S. et al. Distinct synovial tissue macrophage subsets regulate inflammation and remission in rheumatoid arthritis. *Nat. Med.* **26**, 1295–1306 (2020).
124. Cohen, S. B. et al. Denosumab treatment effects on structural damage, bone mineral density, and bone turnover in rheumatoid arthritis: a twelve-month, multicenter, randomized, double-blind, placebo-controlled, phase II clinical trial. *Arthritis Rheum.* **58**, 1299–1309 (2008).
125. Takeuchi, T. et al. Effect of denosumab on Japanese patients with rheumatoid arthritis: a dose-response study of AMG 162 (Denosumab) in patients with RheumatoId arthritis on methotrexate to Validate inhibitory effect on bone Erosion (DRIVE)–a 12-month, multicentre, randomised, double-blind, placebo-controlled, phase II clinical trial. *Ann. Rheum. Dis.* **75**, 983–990 (2016).
126. Takeuchi, T. et al. Effects of the anti-RANKL antibody denosumab on joint structural damage in patients with rheumatoid arthritis treated with conventional synthetic disease-modifying antirheumatic drugs (DESIRABLE study): a randomised, double-blind, placebo-controlled phase 3 trial. *Ann. Rheum. Dis.* **78**, 899–907 (2019).
127. Axmann, R. et al. CTLA-4 directly inhibits osteoclast formation. *Ann. Rheum. Dis.* **67**, 1603–1609 (2008).
128. Blanco, F. J. et al. Secukinumab in active rheumatoid arthritis: a phase III randomized, double-blind, active comparator- and placebo-controlled study. *Arthritis Rheumatol.* **69**, 1144–1153 (2017).
129. Lubberts, E. et al. Treatment with a neutralizing anti-murine interleukin-17 antibody after the onset of collagen-induced arthritis reduces joint inflammation, cartilage destruction, and bone erosion. *Arthritis Rheum.* **50**, 650–659 (2004).
130. Glatt, S. et al. Efficacy and safety of bimekizumab as add-on therapy for rheumatoid arthritis in patients with inadequate response to certolizumab pegol: a proof-of-concept study. *Ann. Rheum. Dis.* **78**, 1033–1040 (2019).
131. Maeshima, K. et al. The JAK inhibitor tofacitinib regulates synovitis through inhibition of interferon-γ and interleukin-17 production by human $CD4^+$ T cells. *Arthritis Rheum.* **64**, 1790–1798 (2012).
132. Kubo, S. et al. The JAK inhibitor, tofacitinib, reduces the T cell stimulatory capacity of human monocyte-derived dendritic cells. *Ann. Rheum. Dis.* **73**, 2192–2198 (2014).
133. Combe, B. et al. Filgotinib versus placebo or adalimumab in patients with rheumatoid arthritis and inadequate response to methotrexate: a phase III randomised clinical trial. *Ann. Rheum. Dis.* **80**, 848–858 (2021).
134. Traves, P. G. et al. JAK selectivity and the implications for clinical inhibition of pharmacodynamic cytokine signalling by filgotinib, upadacitinib, tofacitinib and baricitinib. *Ann. Rheum. Dis.* **80**, 865–875 (2021).
135. Adam, S. et al. JAK inhibition increases bone mass in steady-state conditions and ameliorates pathological bone loss by stimulating osteoblast function. *Sci. Transl. Med.* **12**, eaay4447 (2020).
136. Murakami, K. et al. A Jak1/2 inhibitor, baricitinib, inhibits osteoclastogenesis by suppressing RANKL expression in osteoblasts in vitro. *PLoS ONE* **12**, e0181126 (2017).
137. Matzelle, M. M. et al. Resolution of inflammation induces osteoblast function and regulates the Wnt signaling pathway. *Arthritis Rheum.* **64**, 1540–1550 (2012).
138. Chen, X. X. et al. Sclerostin inhibition reverses systemic, periarticular and local bone loss in arthritis. *Ann. Rheum. Dis.* **72**, 1732–1736 (2013).
139. Marenzana, M., Vugler, A., Moore, A. & Robinson, M. Effect of sclerostin-neutralising antibody on periarticular and systemic bone in a murine model of rheumatoid arthritis: a microCT study. *Arthritis Res. Ther.* **15**, R125 (2013).
140. Teng, Y. et al. Adenovirus-mediated delivery of Sema3A alleviates rheumatoid arthritis in a serum-transfer induced mouse model. *Oncotarget* **8**, 66270–66280 (2017).
141. Zhang, H. et al. NOTCH inhibits osteoblast formation in inflammatory arthritis via noncanonical NF-κB. *J. Clin. Invest.* **124**, 3200–3214 (2014).
142. Sawai, H., Park, Y. W., He, X., Goronzy, J. J. & Weyand, C. M. Fractalkine mediates T cell-dependent proliferation of synovial fibroblasts in rheumatoid arthritis. *Arthritis Rheum.* **56**, 3215–3225 (2007).
143. Laragione, T., Brenner, M., Sherry, B. & Gulko, P. S. CXCL10 and its receptor CXCR3 regulate synovial fibroblast invasion in rheumatoid arthritis. *Arthritis Rheum.* **63**, 3274–3283 (2011).
144. Nanki, T. et al. Inhibition of fractalkine ameliorates murine collagen-induced arthritis. *J. Immunol.* **173**, 7010–7016 (2004).
145. Hamilton, J. A., Cook, A. D. & Tak, P. P. Anti-colony-stimulating factor therapies for inflammatory and autoimmune diseases. *Nat. Rev. Drug Discov.* **16**, 53–70 (2016).
146. Tanaka, Y. et al. Efficacy and safety of E6011, an anti-fractalkine monoclonal antibody, in patients with active rheumatoid arthritis with inadequate response to methotrexate: results of a randomized, double-blind, placebo-controlled phase II study. *Arthritis Rheumatol.* **73**, 587–595 (2021).
147. Yellin, M. et al. A phase II, randomized, double-blind, placebo-controlled study evaluating the efficacy and safety of MDX-1100, a fully human anti-CXCL10 monoclonal antibody, in combination with methotrexate in patients with rheumatoid arthritis. *Arthritis Rheum.* **64**, 1730–1739 (2012).
148. US National Library of Medicine. *ClinicalTrials.gov* https://clinicaltrials.gov/ct2/show/NCT04134728 (2022).
149. Finch, R. et al. Results of a phase 2 study of RG6125, an anti-cadherin-11 monoclonal antibody in

rheumatoid arthritis patients with an inadequate response to anti- TNFα therapy [abstract OP0224]. *Ann. Rheum. Dis.* **78**, 189 (2019).
150. Dorst, D. N. et al. Targeting of fibroblast activation protein in rheumatoid arthritis patients: imaging and ex vivo photodynamic therapy. *Rheumatology* https://doi.org/10.1093/rheumatology/keab664 (2021).
151. Pap, T. & Korb-Pap, A. Cartilage damage in osteoarthritis and rheumatoid arthritis–two unequal siblings. *Nat. Rev. Rheumatol.* **11**, 606–615 (2015).
152. Araki, Y. & Mimura, T. Matrix metalloproteinase gene activation resulting from disordred epigenetic mechanisms in rheumatoid arthritis. *Int. J. Mol. Sci.* **18**, 905 (2017).
153. Posthumus, M. D. et al. Serum levels of matrix metalloproteinase-3 in relation to the development of radiological damage in patients with early rheumatoid arthritis. *Rheumatology* **38**, 1081–1087 (1999).
154. Chang, S. H. et al. Excessive mechanical loading promotes osteoarthritis through the gremlin-1-NF-κB pathway. *Nat. Commun.* **10**, 1442 (2019).
155. Han, E. J. et al. GREM1 is a key regulator of synoviocyte hyperplasia and invasiveness. *J. Rheumatol.* **43**, 474–485 (2016).

Acknowledgements
The authors are grateful to all the laboratory members, especially K. Okamoto, M. Tsukasaki and R. Ling for thoughtful discussion.

Author contributions
The authors contributed equally to all aspects of the article.

Competing interests
The authors declare no competing interests.

Peer review information
Nature Reviews Rheumatology thanks M. Nakamura, J. Lorenzo and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.